

THE
JOURNAL OF
COMPARATIVE PATHOLOGY
AND
THERAPEUTICS.

Vol. XLIX.—No. 4. DECEMBER 31st, 1936.

PRICE 4s.

A STUDY OF IMMUNITY PRODUCED BY INFECTION
WITH ATTENUATED CULTURE-STRAINS
OF *HISTOMONAS MELEAGRIDIS*.

By ERNEST EDWARD TYZZER, M.D.

*Department of Comparative Pathology,
Medical School and School of Public Health,
Boston, Massachusetts.*

IN a previous paper (Tyzzar, 1934) dealing with *Histomonas meleagridis*, the protozoön of "blackhead," there were reported loss of pathogenicity in the course of long cultivation and immunisation through infection with an attenuated culture strain. The degree of pathogenicity of any culture strain of this organism may be readily determined by rectal inoculation of young chickens or turkeys. For this purpose it is advantageous to keep stock birds free of intestinal protozoa through isolation in sterilised cages and the sterilisation of all food material furnished from the time of hatching. Unless such measures are adopted as a matter of routine procedure, intestinal flagellates and parasitic amœbæ are likely to establish themselves in the cæca and thus introduce unnecessary difficulties and confusion. Only chickens of Rhode Island Red stock have been employed for both pathogenicity and protection tests, although it is probable that other breeds would prove to be quite as suitable. Blackhead following the rectal inoculation of *Histomonas* is identical in character with that which occurs spontaneously under farm conditions, except that the incubation period is shortened on account of the large number of organisms which immediately reach the cæca.

The term infection as here employed signifies the establishment of *Histomonas* in the cæca irrespective of whether there is demonstrable invasion of the tissue. Also the term immunity is used to imply either partial or complete protection of the host

against blackhead on inoculation of strains of *Histomonas* known to be pathogenic. It is possible that the protection afforded by the rectal inoculation of *Histomonas* attenuated through long cultivation may be of the type often designated 'premunition.' While it is definitely known that, following the inoculation, the protozoön becomes established in the cæcal contents and in some instances may invade the cæcal wall, all evidence points to the termination of such invasions by its complete elimination from the tissues. Although it seems probable that successive minor invasions may occur, this is at present purely a matter of conjecture, hence the adherence to the more comprehensive term 'immunity'.

EXPERIMENTAL STUDIES.

Attenuation of Virulence during Cultivation.

Tests for pathogenicity have been made at irregular intervals during a period of years, on several strains that have been maintained in mixed culture with bacteria. All strains have been propagated under a routine procedure of weekly transfer in a medium described in a former paper (Tyzzer, 1934).

This medium consists of agar slants covered with 8.5 per cent. aqueous solution of NaCl containing sterile horse serum, a small amount of fine rice starch sterilized by dry heat being added to each tube. While formerly serum to the amount of 5 per cent. of the saline solution was employed, about a year ago this was reduced to 2.5 per cent., which appears to be adequate for the multiplication of *Histomonas* while the growth of bacteria is less profuse. The solid portion of the medium is prepared by heating agar to the amount of 1.5 per cent. in water with 6.6 per cent. sodium chloride and 3 per cent. potassium acid phosphate (KH_2PO_4) and adjusted to pH of 7.2. For the details of the preparation of the agar, the paper referred to above may be consulted. The cultures have been kept for the most part at an incubator temperature of 37.5° C., although there have been periods of weeks or months when somewhat higher temperatures of from 38° to 39° C. were maintained. The stirring of the sediment in the culture favours the development of *Histomonas* to a remarkable degree. Hence, if very rich cultures are desired, the sediment should be broken up and dispersed through the fluid of the medium by gentle churning with a capillary pipette, either several days or at intervals after making a transfer.

No detailed study has been made of the bacteria which are maintained in the *Histomonas* cultures. The impression is that after a time a single bacterial species predominates, although the flora of the culture may change from time to time in the course of long cultivation. While the possibility that the bacteria of the cultures may in some instances be of such a nature as to modify the character or the degree of the protozoan infection has been considered, attempts to demonstrate any such effect have failed, and, as previously reported (1934), it is clear that the loss of pathogenicity in the culture strain tested has its basis in a modification of the biological character of *Histomonas* during cultivation. There is no impairment of infectivity with loss of

pathogenicity in *Histomonas*, since the proportion of successful inoculations is quite as high with the attenuated as with the pathogenic strains. It is also quite certain that, with the former, the carrier state often may be established at once without preliminary invasion of the tissues; for, while even mild inflammation of the cæcal mucosa is associated with great numbers of leucocytes in the cæcal discharges, the daily occurrence in these of *Histomonas* in great number may be unattended with any increase in the number of leucocytes.

The criteria upon which degrees of pathogenicity are distinguished are based on the extent of invasion, the severity of the host's reaction and the duration of the disease. Thus invasion may be confined to microscopic foci in the cæcal mucosa, to localised but macroscopic lesions or there may be generalised invasion of the greater part of the cæcal walls. Metastasis of the infection to the liver is regarded as indicative of greater pathogenicity than cæcal involvement alone. The extent and character of leucocytic infiltrations of the cæcal wall as well as the nature and amount of the exudate accumulating in the cavity of the cæca furnish an index of the severity of the host's reaction.

While outbreaks of blackhead with considerable mortality are occasionally encountered in young chickens, this species is much more resistant than the turkey. Thus, although experimental infections with the more pathogenic strains of the protozoön may produce extensive cæcal involvement and liver lesions in the chicken, the disease is of brief duration and is not frequently fatal. With recovery in this host, *Histomonas* is usually found multiplying in the cæcal contents, the carrier state being maintained for many months, at least under laboratory conditions. In the turkey, however, blackhead tends to be both severe and progressive, so that it is regularly fatal during the first three months of life and thereafter recovery from clinically recognisable disease is infrequent. In conformity with such differences in host reaction to fully pathogenic *Histomonas*, it is found that culture strains of the protozoön that have become quite innocuous for young chickens may be still mildly pathogenic for turkeys.

The results of pathogenicity tests made from time to time are set forth in Table I, which, however, does not include data on the inoculation of young turkeys for purposes of immunisation, which are summarised in Table III. Detailed information concerning the vaccination of young turkeys during the summer of 1932 is furnished in Tables IX and X of an earlier paper. All chickens inoculated were killed within 14 days and with few exceptions between four and ten days after rectal injection of *Histomonas* culture. In turkeys listed in Table I, death from blackhead occurred in from nine to 15 days after inoculation except in one which recovered from the resulting attack but when killed subsequently showed extensive scarring of liver and cæca.

TABLE I.

PATHOGENICITY TESTS OF FIVE *Histomonas meleagridis* STRAINS* IN COURSE OF PROPAGATION IN CULTURE.

Strain.	Date of Test.	Interval after Isolation.	No. of Birds.	Age.	Post mortem Finding.†
A	Dec. 12, 1930	10½ months	4	4 day-old chickens	2 Extensive caecal disease, liver lesions. 2 Extensive caecal disease.
	Jan. 5, 1932	23 months	1 30	"	† No evidence of disease.
	Jan. 23, 1932	23½ months	1 13	"	† No evidence of disease.
	Feb. 6, 1932	24 months	1 45	"	† No evidence of disease.
	Feb. 13, 1932	24½ months	10 19	"	† 10 No evidence of disease.
	Mar. 5, 1932	25 months	5 40	"	No evidence of disease.
	Mar. 28, 1932	25½ months	10 12	"	No evidence of disease. 1 Shows a single and 1 several minute opacities with microscopic evidence of invasion.
	May 17, 1932	27½ months	11 8	"	10 No evidence of disease. 1 A minute opacity in caecum, microscopic evidence of invasion inconclusive.
	July 12, 1932	29½ months	3 12	"	3 No evidence of disease.
	May 26, 1933	39¾ months	10 1	"	9 No evidence of disease. 1 Slight thickening of caecal walls.
	May 23, 1935	63½ months	5 14	"	5 No evidence of disease.
	Mar. 5, 1932	7 days	5 40	"	5 Extensive caecal disease.
	Mar. 10, 1932	12 days	4 45	"	1 Extensive caecal disease, liver lesions. 3 Extensive caecal disease.
B	Mar. 24-29, 1932	26-31 days	5 8-13	"	1 Extensive caecal disease, liver lesions. 3 Extensive caecal disease.
	Mar. 26, 1932	28 days	5 10	"	1 No infection. 3 Extensive caecal disease, liver lesions.
	May 27, 1932	2 months	1 17	turkeys	2 Extensive caecal disease. Death with extensive caecal disease and liver lesions.
	June 4, 1932	2¼ months	1 25	"	2 Deaths with extensive caecal disease and liver lesions.
	June 6, 1932	2½ months	2 27	"	2 Deaths with extensive caecal disease and liver lesions.
	July 27, 1932	5 months	2 28	"	2 Deaths with extensive caecal disease and liver lesions.
	Oct. 1, 1932	7½ months	2 59	"	1 Death with extensive caecal disease and liver lesions.
	May 26, 1933	15 months	6 1	chickens	1 Recovery with extensive scarring of caeca and liver. 6 No evidence of disease.
	June 1, 1933	16½ months	9 4	turkeys	1 Extensive caecal disease.
	June 1, 1933	16½ months	9 4	turkeys	1 Extensive caecal disease.
	Isolated on Feb. 27, 1932, from a chicken fed Heterakis ova 7 days previously				

7 Slight caecal disease.

1 No infection.

4 No evidence of disease.

1 Caecal walls thickened.

4 No evidence of disease.

D

Isolated on Aug. 3, 1932,
from a 34-day turkey
fed embryonated Heter-
akis ova 7 days previ-
ously

July 17, 1933	16 $\frac{2}{3}$ months	5 28	"	chickens	4 Extensive caecal disease, liver lesions.
Apr. 24, 1935	38 months	4 15	"	"	2 Extensive caecal disease.
May 26, 1933	9 $\frac{2}{3}$ months	6 1	"	"	4 Extensive caecal disease, liver lesions.
June 24, 1933	10 $\frac{2}{3}$ months	8 30	"	"	4 Extensive caecal disease, liver lesions.
Apr. 15, 1935	32 $\frac{1}{3}$ months	5 6	"	"	1 Extensive caecal disease, liver lesions.
July 3, 1935	35 months	5 15	"	"	4 Extensive caecal disease.
			"	"	3 Extensive caecal disease, liver lesions.
			"	"	2 Extensive caecal disease.

E

Isolated on Aug. 16, 1934,
from liver lesions of a
turkey dying of black-
head

Sept. 26, 1934	41 days	2 14	"	"	1 Localized caecal disease.
Feb. 25, 1935	6 $\frac{1}{3}$ months	8 13	"	"	1 Blood-tinged exudate from caecum, not killed.
Mar. 27, 1935	7 $\frac{1}{3}$ months	6 42	"	"	7 Extensive caecal disease, liver lesions.
May 8, 1935	8 $\frac{2}{3}$ months	4 29	"	"	1 Extensive caecal disease.
			"	"	5 Extensive caecal disease, liver lesions.
			"	"	1 Extensive caecal disease.
			"	"	3 Extensive caecal disease, liver lesions.
June 1, 1935	9 $\frac{1}{2}$ months	6 23	"	"	1 No lesion, <i>Histomonas</i> present (killed 12 days after inoculation).
			"	"	2 Extensive caecal disease, liver lesions.
			"	"	4 (Killed within 5 days) caecal disease.

F

Isolated on June 27, 1935,
from caecal exudate of
an adult quail showing
chronic caecal blackhead

June 28, 1935	1 day	2 10	"	"	1 Extensive caecal disease, liver lesions.
June 29, 1935	2 days	1 11	"	"	1 Extensive caecal disease.
			"	"	Extensive caecal disease, liver lesions.

* Strain C not tabulated on account of some uncertainty as to exact origin (derived from a 22-day old turkey exposed for several days to brooder-house conditions and also with a possibility of exposure in the laboratory to Strain A infected turkeys of the same age), and the small number of birds upon which it was tested. Indications were that it was, immediately after isolation in culture, non-pathogenic for chickens, slightly pathogenic for young turkeys.

† Unless specifically recorded as "No infection," *Histomonas* was shown by microscopic examination to be established in the caecal contents of each inoculated bird.

‡ Inoculated with caecal discharges containing Strain A *Histomonas* in attempt to restore virulence by successive bird-to-bird passage.

TABLE II.
THE IMMUNIZING PROPERTIES OF DIFFERENT STRAINS OF *Histomonas meleagridis* AFTER VARIOUS PERIODS UNDER CULTIVATION.

Chicken.	Hatch.	Immunizing Strain* Time under Cultivation.	Immunizing. Time under Cultivation.	Test Strain† Time under Cultivation.	Test-Inoculation.	Degrees of Protection when Killed.‡
8533 8534 8535	Dec. 23, 1931	A§, about 24 months	Jan. 20, 23, 28 and Feb. 6, 1932	B, direct from caecal discharge of chicken from which Strain B was cultivated	Mar. 4, 1932	Complete at 11 days. Almost complete at 13 days. Almost complete at 14 days.
8540 8541 8543 8544	Dec. 23, 1931	A, about 24 months	Jan. 20, 23, 28 and Feb. 6, 1932	B, 9 days	Mar. 5, 1932	Almost complete at 6 days. Almost complete at 7 days. Complete at 5 days. Partial at 9 days. Almost complete at 10 days.
8555	Jan. 25, 1932	A, 25 months	Mar. 5, 1932	B, 19 days, also caecal discharges of Strain B infected chicken	Mar. 15, 1932	Complete at 6 days.
8582 8583 8584	Mar. 16, 1932	A, 25½ months	Mar. 19, 1932	Embryonated Heterakis ova, collected on Feb. 13, 24 and 29, kept in 1.5 per cent. nitric acid	Apr. 1, 1932	Complete at 8 days. Almost complete at 8 days. Complete at 10 days.
8756 8757 8758 8759 8760 8761 8762	June 30, 1932	A, 29 months	July 12, 1932	B, 6 months and D, 24 days	Aug. 27, 1932	Complete at 7 days. Complete at 10 days. Almost complete at 7 days. Almost complete at 10 days. Complete at 10 days. Partial at 10 days. Partial at 10 days.
8851 8852 8853 8854 8855 8856 8857	May 25, 1933	A, 30¾ months	May 26, 1933	D, 10¾ months	June 24, 1933	Slight at 6 days. Partial at 6 days. Slight at 6 days. Partial at 6 days. Complete at 6 days. Slight at 6 days. Slight at 6 days.
9121 9122 9123	Feb. 12, 1935	A, 61 months	Mar. 8, 1935	E, 7¼ months, and caecal discharges of Strain E in- fected chickens	Mar. 27 and 29, 1935	Inconclusive at 3 days. None at 4 days. None at 5 days.

9124						Partial at 6 days.
9125						Partial at 6 days.
9126						None at 7 days.
9157	May 9, 1935	B, 38½ months	May 15, 1935	E, 9½ months	June 1, 1935	Inconclusive at 2 days.
9158						Partial at 3 days.
9159						Slight at 4 days.
9160						Slight at 5 days.
9161						Partial at 6 days.
9162						Partial at 7 days.
9163						Partial at 9 days.
9164						Partial at 10 days.
9176	May 9, 1935	B, 39½ months	June 1, 1935	D, 34½ months	June 20, 1935	Almost complete at 5 days.
9177						Almost complete at 7 days.
9179	June 18, 1935	B, 39½ months	June 19, 1935	D, 35 months	July 3, 1935	Almost complete at 10 days.
9180						Complete at 6 days.
9181						Partial at 7 days.
9182						Partial at 8 days.
9183						Complete at 5 days.
9137	Apr. 9, 1935	D, 32½ months, pathogenic	Apr. 15, 1935	E, 8½ months	May 8, 1935	Complete at 12 days.
9138						Complete at 12 days.
9141						Complete at 7 days.
9142						Complete at 7 days.
9143						Complete at 7 days.
9144						Complete at 7 days.
9145						Complete at 12 days.
9113	Feb. 12, 1935	E, 6½ months, pathogenic	Feb. 25, 1935	E, over 7½ months (and cecal discharge of Strain E infected chicken)	Mar. 27, 1935	Complete at 5 days.
9114						Complete at 3 days.
9119						Partial at 6 days.
9120						Almost complete at 4 days.

*Non-pathogenic except when otherwise indicated.

†Pathogenic in all cases.

‡Complete protection signifies no pathological finding at autopsy of the vaccinated bird at an appropriate interval after the immunity test-inoculation; *almost complete*, slight evidence of disease, caeca functioning; *partial*, foci of disease markedly less extensive than in control; *slight*, less extensive disease than in control but without great contrast; and *no protection* indicating disease quite as extensive in the vaccinated as in the control bird.

§For protective effect of Strain A inoculation of young turkeys in May, June and August, 1932, see Table IX, Tyzzer, 1934.

||Inoculated with cecal discharges of chickens infected with Strain A, except chicken 8756 which was inoculated with culture.

TABLE III.

SUMMARY OF RESULTS OBTAINED IN THE VACCINATION OF TURKEY POULTS AGAINST BLACKHEAD IN FOUR CONSECUTIVE SUMMERS.

Year.	Number of Poults.	Vaccination by Rectal Inoculation.	Deaths* following Vaccination.	Immunity Tests.	Subsequent Deaths from Blackhead.
1932	15	Strain A at ages of 7 to 14 days	0	Strain B inoculated 17 to 20 days after vaccination	1
	4	(Controls)	—	Strain B inoculated on same date as preceding group	4
	2	Strain A at ages of 2 and 6 days	0	Strain B inoculated 21 and 31 days after vaccination	0
	2	(Controls)	—	Strain B inoculated on same date as preceding two	1†
	2	Strain A at age of 2 days	0	Embryonated Heterakis ova fed 25 days after vaccination	0
	2	(Controls)	—	Embryonated Heterakis ova fed on same date as preceding two	2
	7	Strain A at ages of 3 to 55 days	0	Strain B twice inoculated between 14 and 56 days after vaccination	0
	26	Strain A at ages of 3 to 25 days	7‡	Exposed to farm conditions after intervals of 0 to 22 days after vaccination Strain B and D inoculated 103 to 125 days after vaccination	2 0
	25	Strain A at ages of 3 to 15 days	3§	Exposed to farm conditions after intervals of 2 to 3 weeks after vaccination	0
Totals 1932	77	(Controls not included)	10		3
1933	50	Strain A on from 1 to 3 occasions at ages of 3 to 18 days	0	Strain B inoculated 24 to 29 days after first vaccination	3
1934	50	Strain A at ages of 3, 7 and 17 days	1¶	Strain B inoculated 1 month after vaccination Strain D inoculated 2 months after vaccination	2 1
1935	43**	Strain B at ages of 1 to 6 days	0	Strain D inoculated 11 to 16 days after vaccination	13

*Deaths from all causes included.

†The other control suffered a severe and protracted attack of blackhead but recovered. When killed two months after inoculation, liver and cæca showed extensive scarring.

‡Two died and five were killed, all showing blackhead lesions but confined with one exception to the cæca. The poults of this group were taken from a poor hatch following a brief but great elevation of incubator temperature and showed evidence

All strains of *Histomonas* thus far isolated in culture, with the exception of Strain C not tabulated on account of doubt as to origin, were at first markedly pathogenic. It is apparent that Strain A became innocuous for young chickens within a period of two and a half years from the time of isolation. It also had lost in this period most of its pathogenesis for young turkeys, as may be seen from the results of Strain A inoculations presented in Table III. Strain B had become relatively non-pathogenic for young chickens and turkeys before the end of one and a half years. In contrast, the third strain, D, was still pathogenic for young chickens almost three years after its isolation. There are thus obvious differences in the rate at which this biological change in *Histomonas* takes place.

Immunisation through Rectal Inoculation of Histomonas Cultures.

The protective effect of the inoculation of chickens with culture strains of *Histomonas* that had lost much of their pathogenicity have also been determined from time to time, usually by subsequent inoculation with more recently isolated and more virulent strains. The degree of protection derived from the vaccination inoculation has been ascertained by comparison of the cæca of the vaccinated series with those of control non-vaccinated series, taken at appropriate periods after test inoculation with virulent *Histomonas*. While the control series appear in Table I, the data pertaining to the vaccinated series are presented in Table II. Rectal injection was employed for both vaccination and test inoculation except when otherwise indicated.

Strain A, after becoming non-pathogenic, furnished for a period of nearly six months in 1932 either complete or almost complete protection to chickens against virulent cultures of *Histomonas*. The immunising property of this strain was subsequently reduced, however, so that by the summer of 1933, slightly over three and a quarter years after its isolation, slight, partial or complete protection resulted from its inoculation. Later on, in tests made somewhat over five years from the time of its isolation, it was shown to possess at this time only slight protective properties.

of lowered resistance in unusual complications, such as pericarditis and peritonitis of bacterial origin in association with localised *Histomonas* invasion.

§ In one of these poults, death was attributable to a diverticulum of the gizzard and showed only microscopic foci of *Histomonas* invasion; another, a runt with large unabsorbed yolk mass, died of peritonitis and showed only localised *Histomonas* invasion of the cæca; while the third poult died of typical and progressive blackhead infection.

|| Two others died before maturity without lesions indicating blackhead.

¶ Death due to acute peritonitis, no evidence of invasion of the tissues by *Histomonas*.

** There was a high early mortality in this lot of poults. Nine that died within a week from the time of hatching are not included in this figure.

Strain B, which within a year and a half of its isolation showed a marked, although not total, loss of pathogenicity for young chickens and turkeys (*see* Table I), after being under cultivation over three years, produced various degrees of immunity in chickens, ranging from slight to complete protection.

Strain D, still mildly pathogenic for chickens nearly three years after isolation, at this time completely protected against a more recently isolated and virulent Strain E. The test of the immunising properties of the latter strain towards itself yielded in a group of four chickens an unexpected result, one showing extensive blackhead, evidently the result of the second inoculation, and another bird showing a limited invasion of the cæca.

For four successive summers attenuated cultures were employed in attempts to vaccinate young turkeys against blackhead. While all except the earlier inoculations were undertaken for commercial rather than for scientific purposes, the results may be of interest and are summarised in Table III. *Histomonas* is not always established in the cæca of the young turkey by a single inoculation of culture material, so that it is preferable to examine the cæcal discharges of each bird for the protozoon and to reinoculate at intervals until infection is obtained. *Histomonas* was demonstrated in the cæcal discharges of every turkey vaccinated in the seasons of 1932 and 1935, but in 1933 and in 1934 this could not be readily accomplished and reliance was placed on reinoculation and continuous exposure to the infected members of the flock. The time factor has to be taken into account and it is inadvisable to keep turkeys intended for market caged indoors for too long a time.

These results indicate a considerable degree of protection afforded by the establishment of an attenuated strain of *Histomonas* in the cæca of young turkeys for three consecutive years. The following year, 1935, Strain B failed to furnish prompt, adequate protection, so that twelve of the thirteen deaths may be attributed to the inoculation of Strain D, which was made rather for the purpose of augmenting the immunity afforded by the previous vaccination than as a test for its presence. There is some question as to the immunising properties of Strain B at this time, for it had not afforded complete protection in chickens (*see* Table II). The pathogenicity of Strain D for turkeys was under estimated and the latter also was probably inoculated too soon to obtain the full benefit of whatever protection the Strain B vaccination would afford.

Since the exposure of turkeys early in life to fully pathogenic *Histomonas* infection would doubtless result in an almost 100 per cent. mortality, the effects of vaccination by attenuated culture strains appear significant. In regard to immediate effects of vaccination, the results suggest that when carried out under favourable conditions it may be unattended with any serious

consequence, but that in stock that is weak or in any way abnormal this procedure tends to increase mortality. With evidence indicating that loss of infection may be followed by loss of immunity, it is probably advantageous to keep vaccinated turkeys under conditions in which they will be continuously exposed to infective material in order that the carrier state may be maintained.

While the immunisation of turkeys against blackhead is possible under well-controlled conditions, apparently insurmountable obstacles interfere with the utilisation, in the commercial rearing of turkeys, of the vaccination procedure here employed. These obstacles have been discussed in a previous paper (Tyzzer, 1934).

The gradual diminution in both extent and intensity of the pathological process, which accompanies loss of pathogenicity, lends support to the hypothesis previously offered, namely, that the loss of immunising properties which subsequently follows is based on further loss of invasive power in *Histomonas*. Occasional foci of invasion of the mucosa have been demonstrated in sections of cæca which appear normal to the naked eye. Such microscopic foci were found in series of birds infected with attenuated culture strains that were, at the time, seldom producing gross evidence of disease, and, at most, no more than localised areas of thickening of the cæcal wall. It seems quite probable that the optimum protection is derived by localised invasion followed by the establishment of the carrier state, but that, with extensive involvement, the reaction may be so severe that *Histomonas* is eliminated from the cæca. In the latter case, protection apparently is either less adequate or of brief duration. Thus chicken No. 9119 (Table II), inoculated with a virulent strain, reinoculated with the same strain four and a half weeks later, and killed after an additional period of six days, showed a moderate degree of acute infection in cæca which were thickened and scarred from the first infection. Of three other similarly treated chickens, two showed complete and one almost complete protection.

HISTOLOGICAL STUDIES.

A comparative study of the tissue reactions in the cæca of chickens immune, partially immune, and non-immune to *Histomonas* invasion was made in the period between February 25th and August 2nd, 1935, employing five culture strains. The tissue reactions obtained in test inoculations of a series of vaccinated birds may be compared with those of control series inoculated with the strains of *Histomonas* used in vaccination and in test inoculations respectively. Chickens for comparative series were always of the same stock and from a single purchase lot, were kept under identical conditions and were frequently of the same age when inoculated. The chicken has been employed in this study chiefly on account of its availability. It is quite possible

that the turkey, in which the tissue reactions to *Histomonas* are less pronounced, would furnish a somewhat different picture. The various inoculations which furnished the material for this study together with certain related data are outlined in Table IV.

TABLE IV.

SOURCES OF MATERIAL FOR HISTOLOGICAL STUDY: (a) FOR COMPARISON OF THE REACTIONS OF NORMAL CHICKENS TO DIFFERENT CULTURE STRAINS OF *Histomonas*; (b) FOR COMPARISON OF THE REACTIONS OF NORMAL CHICKENS WITH THOSE PROTECTED TO SOME DEGREE BY PREVIOUS INOCULATION, TO PATHOGENIC STRAINS OF *Histomonas*.

Lot of Chicken.	Primary Inoculation.	Secondary Inoculation.	Number of Chickens.	Age at last Inoculation.	Killed after Last Inoculation.
R.I.R. hatched Feb. 12	Strain E Feb. 25	None	8	13 days	7, 7, 7, 7, 8, 8, 9 and 9 days
	Strain E Feb. 25	Strain E Mar. 27	4	43 days	3, 4, 5 and 6 days
	Strain A Mar. 8	Strain E Mar. 27	6	43 days	(3)*, 4, 5, 6, 6 and 7 days
	Strain E Mar. 27	None	7	43 days	3, 4, 5, 6, 6, 7 and 8 days
R.I.R. hatched Apr. 9	Strain D Apr. 15	None	5	6 days	5, 7, 7, 9 and 9 days
	Strain D Apr. 15	Strain E May 8	7	29 days	(7), (7), (7), (7), (12), (12), and (12) days
	Strain E May 8	None	4	29 days	7, 7, 12 and 12† days
	Strain B Apr. 24	None	4	15 days	(6), (8), (8) and (9) days
R.I.R. hatched May 9	Strain B May 15	Strain E June 1	8	23 days	(2), 3, 4, 5, 6, 7, 9 and 10 days
	Strain E June 1	None	6	23 days	2, 3, 4, 5, 6 and 7 days
	Strain A May 23	None	5	14 days	(4), (5), (6), (9) and (11) days
	Strain B June 1	Strain D June 20	2	42 days	4 and 7 days
R.I.R. hatched June 18	Strain B June 19	Strain D July 3	5	15 days	(5), (6), 7, 8 and (10) days
	Strain D July 3	None	5	15 days	5, 6, 7 (8) and 10 days
	Strain F June 28	None	3	10 days	5, 7 and 7 days
	Strain B July 18	None	3	30 days	(2), (3) and (4) days
	Strain B July 18	Strain D Aug. 2	3	45 days	(2), (3) and (4) days
	Strain D July 18	None	3	30 days	(2), 3 and 4 days
	Strain D July 18	Strain E Aug. 2	3	45 days	(2), (3) and (4) days

*Numbers in parentheses indicate that in the bird killed on that day there was no evidence of invasion of the tissues by *Histomonas*.

†Recovered before it was killed.

At the time that these inoculations were being made the various culture strains showed obvious differences in pathogenicity. Strains A and B were then wholly non-pathogenic for young chickens, Strain D was mildly pathogenic, and Strains E and F fully pathogenic. Before discussing the differences in the gross and microscopic reactions of normal or immunised chickens to the pathogenic strains, it may be well to describe in a general way the histopathology of the disease process found to involve the cæca of the chicken following the rectal inoculation of *Histomonas*.

Histopathology of the Typhlitis of Blackhead in the Chicken.

The pathological process shows certain essential features which are present in variable degrees with all three strains now showing pathogenicity. As previously pointed out in a study of the disease in turkeys (Tyzzer, 1919), there is a marked change in the morphology of the tissue forms of *Histomonas* during the course of the infection. During active invasion, that is, either in the initial stages of the infection or in the advancing border of lesions, there are relatively large, amœboid organisms with cytoplasm which appears reticular in stained preparations, and contains a considerable amount of ingested particulate material. Following the multiplication of the protozoön within the tissues, the latter become infiltrated with great numbers of closely packed organisms which are in general clear and devoid of inclusions. Such forms present little more than outlines with here and there a distinguishable nucleus. Later in the process, and in association with a monocytic reaction which tends to fix and enclose the protozoön, smaller and denser forms of the latter occur. Probably on account of continued division after being surrounded by the monocytes, the dense forms tend to occur in nests of from several up to a dozen or more. Whereas such dense and readily stained forms are easily recognised in stained sections, forms occurring earlier in the process are only brought out under favourable fixation and staining, and might readily be overlooked by one not familiar with the histology of the disease at all stages. It is not clear to what extent the early occurring forms have been recognised by others; at least they are not distinguishable in Theobald Smith's descriptions, which, except for a brief discussion of degenerating organisms, apply to the more obvious denser forms of the protozoön (Smith, 1895 and 1915). It is quite possible that some of the doubt harboured in regard to the aetiology of this disease is based on failure to recognise those forms of *Histomonas* which occur in early lesions.

Invasion of the mucosa takes place very promptly after rectal inoculation with pathogenic strains of *Histomonas*. Within 48 hours, numbers of the protozoön are seen between the fundi of the glands in association with an intense infiltration with polymorphonuclear leucocytes. The organisms, mingled as they are

with the leucocytes, are by no means conspicuous, and it requires careful examination at this stage of the infection to distinguish them. The polymorphonuclear infiltration appears to be the chief characteristic of the initial stage of the infection in chickens. Subsequently the picture changes and with the multiplication of *Histomonas* there is extensive infiltration with monocytes which gradually check the migration of the protozoa by confining them within spaces among the cells. Under such conditions the organisms may continue to divide for a time, but they do not thrive and soon disappear. Thus while in the early stages of infection the invading organisms call out a polymorphonuclear infiltration, later on they are found in association with monocytes. The latter cells infiltrate all layers of the cæcal wall and extend far beyond the portions actually invaded by *Histomonas*. There also is often considerable œdema, especially prominent in the sub-mucosa. In the course of the reparative process following the disappearance of *Histomonas* the monocytes and lymphoid cells may continue to increase for a time and, with repair, structures having the general appearance of small lymphoid follicles develop in the mucosa.

It is difficult to account for the difference in the initial reaction from that which follows. It is possible that the former is due to bacteria or their products introduced through the migration of numbers of the protozoön from the cæcal contents into the tissues. At least in stained sections, the free forms of *Histomonas* regularly show ingested bacteria whereas the tissue forms seldom contain inclusions of this nature. Doubtless there is a quantitative factor concerned in the severity of the process, in that a more rapid multiplication of invading forms of the protozoön results in more extensive injury. During the active phase of the process *Histomonas* may be present in such numbers as to interfere with the nutrition of the tissues, small blood vessels are frequently occluded, and hæmorrhagic areas appear. In the more severe cases the acute reaction may increase to such an extent that the damaged and often extensively ulcerated mucosa is infiltrated throughout with polymorphonuclear leucocytes. The outpouring of fibrino-purulent exudate may produce thick adherent membranes or, through successive deposits, form laminated sequestra or 'cores' which distend the diseased cæca. The giant cells frequently found after the third day of the infection are derived from the fusion of infiltrating monocytes. They are commonly found arranged around small collections of pus or in a continuous, palisade-like layer against the accumulation of exudate which to a variable extent replaces the mucosa. They frequently ingest numbers of *Histomonas*, but this is regarded as more or less incidental to their attack on exudate and cellular débris.

No conclusion has been reached as to the exact mechanism through which *Histomonas* is destroyed in the tissues. Many of

the protozoa are ingested not only by giant cells but also by the monocytes. Great numbers not so ingested undergo degeneration, so that they appear as ill-defined vesicles, in which the characteristic small nucleus is only occasionally recognisable. In blackhead infection of the chicken, the disappearance of *Histomonas* from the tissues marks in a general way the beginning of the reparative process. A line of demarcation develops between the living tissue and the mingled necrotic tissue and exudate, these latter being incorporated into the cores forming within the cæca. The growth of blood vessels and connective tissue furnishes a granulation tissue in the more seriously damaged areas, while at the same time there is regeneration of the mucous glands. The injury to the cæca in some cases is of such a nature that the reparative process results in pronounced scarring, or the cæcal mucosa may show cystic glands and an increase in connective tissue long after recovery from the acute process. Usually, however, repair is so perfect that the cæca of the recovered bird cannot be distinguished from those of a normal one.

Reactions of Non-immune Chickens to Different Culture Strains.

Strains A and B.—In the present histological study no evidence is obtained of invasion of the cæcal walls of young chickens rectally inoculated with *Histomonas* of either Strain A which has been carried in culture for five years or Strain B under cultivation for three years, notwithstanding that the protozoön was demonstrated in the cæcal contents of each bird of the series studied. In stained sections a few minute foci of polymorphonuclear infiltration are found in the cæcal mucosa of some of the Strain A-inoculated chickens, but no *Histomonas*. In previous studies, made before this strain had completely lost its pathogenicity, infection was found to be limited to minute, even microscopic foci of invasion.

Strain D.—This strain, after being maintained in culture for nearly three years, is mildly pathogenic for young chickens, producing in them cæcal lesions with great regularity and also evidence of metastasis to the liver in about 50 per cent. of cases. Disease resulting from this strain is of brief duration, usually being at its height on or before the sixth day from the inoculation, after which there is prompt recovery. The extent of the cæcal involvement varies, but there are usually localised lesions rather than a generalised inflammation of the cæcal wall. The manifestations of exudation and ulceration are not uniform, but in general give evidence of a relatively mild and transient inflammatory process so that in most cases the cæca function throughout the attack, while repair is prompt and complete. The polymorphonuclear leucocytes, present in considerable numbers in the initial stages of the infection, occur in relatively small numbers in the process that follows. *Histomonas* never occurs in great abundance

and its numbers begin to decrease early, *i.e.*, by the fifth or sixth day, while at the same time the monocytic reaction continues to increase and reaches its greatest intensity following the disappearance of the protozoön. Giant cells constitute a prominent feature in the reaction to Strain D and are regularly abundant from the third or fourth day. There are occasional minute hæmorrhages, but the amount of exudate is small and such ulcers as may occur are of small size and often microscopic.

Strain E.—This, after being under cultivation for nine and a half months, produces regularly in chickens a uniform involvement of the entire saccular portion of the cæca and also liver metastasis in the majority of cases. The infection is severe, being progressive over a longer period than in the case with Strain D and showing more extensive and more serious injury to the cæca. *Histomonas* continues to multiply until enormous numbers are present. Excessive exudation results in the formation of large adherent cores which occlude the cæca. There is destruction of the mucous membrane, with extensive ulceration which does not readily heal. The cæcal walls are rendered non-contractile, they become thickened, rigid and more or less opaque, and gangrene of the entire cæcum may occur. The cæca of recovered chickens are frequently distorted by extensive scars. Microscopically the polymorphonuclear infiltration is excessive and constitutes a prominent feature throughout the active phases of infection, *i.e.*, up to the seventh day. Masses of *Histomonas* bring about local interference with tissue nutrition and there is blocking of small vessels of the submucosa with attendant necrosis. The outpouring of fibrino-purulent exudate from the damaged mucosa assumes massive proportions and small abscesses occur frequently in the submucosa. Giant cells are not commonly found until late in the process, when they may occur in a layer around masses of exudate.

Strain F.—This strain was fully pathogenic immediately after its isolation in culture. From the histological study of only a few cases, it appears to produce a pathological process quite similar to that seen with Strain E.

Thus, to summarise, the comparison of the reactions of chickens to the various culture strains of *Histomonas* shows certain distinctive differences as well as common features. As already pointed out, there is an initial polymorphonuclear infiltration which follows invasion of the mucosa by *Histomonas*. This early reaction around the protozoön is less intense in Strain D than in Strain E. The protozoön subsequently multiplies in the course of Strain D infections, but never to the extent observed with Strain E. In the former there soon appears an intense monocytic infiltration which for the most part replaces the initial polymorphonuclear infiltration and which increases even after the infection is reduced to a few scattered organisms. *Histomonas* is seldom seen in its dense rounded form, apparently being destroyed

too promptly for these forms to develop. It is frequently completely eliminated by the seventh day after inoculation.

In Strain E infection the multiplication of *Histomonas* is rapid and continues for several days longer than in Strain D. During the active phase of the process the tissues are distended with great numbers of organisms. While the monocytes appear in great numbers in association with the elimination of *Histomonas*, this aspect of the process is quite overshadowed by the intense polymorphonuclear infiltration and excessive exudation until repair is well under way. The injury to the mucosa is extensive, and often leads to a condition of general ulceration.

Reaction of Completely and Partially Protected Chickens to Pathogenic Strains of Histomonas.

Complete protection is assumed in vaccinated birds which show no invasion of the tissues following a test inoculation with a pathogenic strain. The cæca of such birds appear either normal or show changes attributable to the vaccinating inoculation, that is, increase of collagen, local collections of lymphoid cells and monocytes, or occasionally cystic distension of the mucous glands. For example, chickens previously inoculated with Strain D are completely protected against Strain E and show no microscopic evidence of invasion following the injection of the latter. When non-pathogenic strains such as A and B are inoculated for purposes of immunisation, a variable degree of protection is obtained, even though at present it is not possible to demonstrate invasion of the tissues by either of these two strains. In such cases, following the test inoculation with pathogenic strains D and E, certain birds of the series may show foci of invasion of the cæcal wall. While the lesions are then much less extensive than in the control bird inoculated with the pathogenic strain, a study of stained sections shows that the tissue reactions of both are in all essentials similar in character. Thus the initial polymorphonuclear reaction is no more intense in partially protected than in unprotected chickens, and the process shows microscopically at least the characters of an unmodified infection.

DISCUSSION.

From these histological studies it is clear that the chicken's reaction to a mildly pathogenic strain of *Histomonas*, as, for example, Strain D, may show essential differences from its reaction to fully pathogenic strains such as Strain E and F. On the other hand, the tissue reactions of partially protected chickens to pathogenic *Histomonas* is of similar character to those observed in birds not previously vaccinated. There are, however, unmistakable gross evidences of protection attributable to vaccination. Some birds of the vaccinated series killed at the time when the controls are approaching the height of the infection show neither

gross nor microscopic evidence of invasion and hence may be regarded as completely protected. In others, taken at a time when the controls show an actively extending infection, the process has reached the stage of repair, *i.e.*, early recovery. Occasionally, vaccinated birds killed rather late present minute lesions representing early stages in the disease process which may be interpreted as evidence of delayed invasion. As compared with the extensive involvement of the cæca of the controls, those vaccinated birds which present lesions show in general a localisation of the process to small areas, often so minute as to be visible only with the hand lens, and requiring verification in stained sections. Even vaccination with Strain A, which has been kept in culture longer than any other strain, may in occasional cases appreciably modify infection with the more recently isolated strains. Thus the gross findings furnished by the partially immunised series, *i.e.*, the total absence of lesions in some individuals, delayed infections, early recovery and limitation of invasion as shown by the localisation of the disease process to small areas, are much more impressive than the histological picture which shows a process that differs only in its extent from that seen in the unprotected control bird.

Infective material derived directly from cases of spontaneous blackhead from many different sources has been found to be regularly pathogenic, although on a single occasion the breast inoculation of a series of young turkeys with liver-lesion material from a spontaneous case of blackhead produced a rather mild, and for the most part non-progressive disease. Infections following the ingestion of many different samples of embryonated *Heterakis* eggs have been likewise regularly associated with extensive disease. However, attempts to bring back by serial passage through young chickens and turkeys the pathogenicity, lost in the course of cultivation, of a strain of *Histomonas* failed completely (Tyzzer, 1934).

SUMMARY.

Various culture strains of *Histomonas meleagridis* show no uniformity in regard to the rate of decrease in pathogenicity. Thus while pathogenicity was lost in one and a half years in one strain, another strain was still mildly pathogenic after three years.

Strains which have become non-pathogenic retain for a time well-marked immunising properties when established in the cæca, but may gradually lose these properties and later on furnish no more than slight or partial protection against inoculation with fully pathogenic strains.

Histological study of successive stages of the pathological process resulting from the invasion of the cæcal wall by *Histomonas* has revealed qualitative as well as quantitative differences in the reaction of the chicken to different culture strains. On comparing the reaction of partially immunised and those of

non-immunised chickens to pathogenic *Histomonas*, the lesions are found to be microscopically similar, differing only in extent. In birds completely immunised, as judged by gross appearances when killed at appropriate periods after the test inoculation, no invasion of the tissues has been demonstrated microscopically.

Notwithstanding the lack of distinctive histological differences in the character of the reactions of the partially protected chickens as compared with those of controls, the modification of the infection in the former is clearly shown in the shortening of the course of infection, in the occurrence of delayed infections, and in the marked limitation of the area of the cæcal wall invaded.

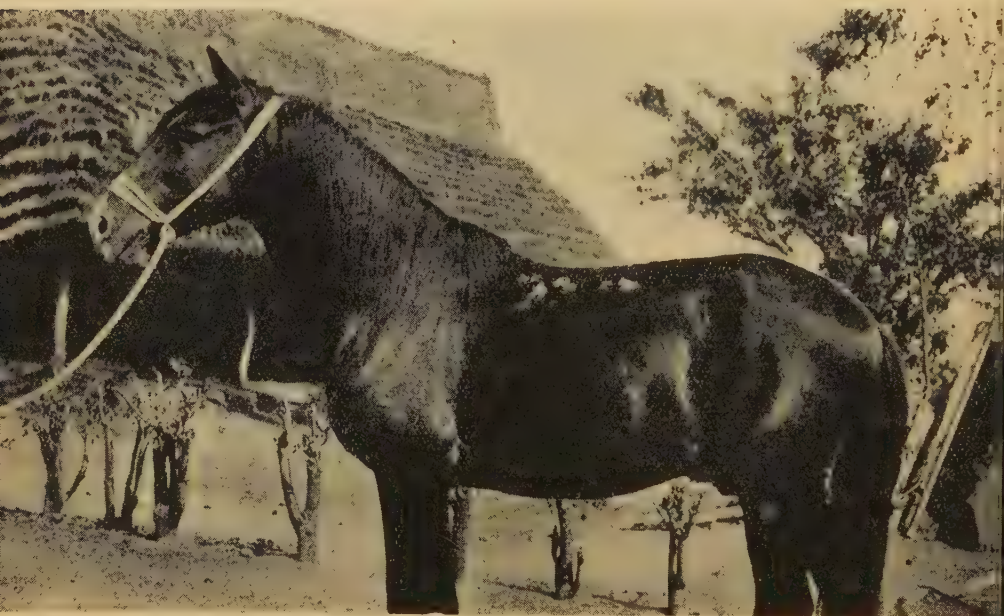
REFERENCES.

- Smith, Theobald. (1895). An infectious disease among turkeys caused by the protozoa (infectious entero-hepatitis). *U.S. Dept. of Agric. Bulletin*, No. 8, 7.
- . (1915). Further investigations into the etiology of the protozoan disease of turkeys known as blackhead, entero-hepatitis, typhlitis, etc. *Jl. Med. Res.*, xxxii, 243.
- Tyzzer, E. E. (1919). Developmental phases of the protozoan of "Blackhead" in turkeys. *Jl. Med. Res.*, xl, 1.
- . (1934). Studies on histomoniasis, or "Blackhead" infection, in the chicken and the turkey. *Proc. Am. Acad. Arts & Sciences*, lxix, No. 5, 189.

TOLERATION OF NAGANOL (BAYER 205) BY HORSES.

By S. C. J. BENNETT, D.SC., M.R.C.V.S.,
Veterinary Research Officer, Sudan Government.

As indicated by the title, this short note, although dealing with equine trypanosomiasis, is written more with the object of demonstrating the extent to which some horses will tolerate repeated injections of Naganol (Bayer 205) than of discussing the curative value of the drug.



Horse DARB. Sudan country-bred stallion, aged 22 years. Has lived in a tsetse fly infested area for five years, having probably been infected with *T. brucei* the whole time. During this period at least 180 injections of 2 gm. Naganol have been given. Photograph by Capt. J. K. Maurice, M.B.E., M.C., Sudan Political Service.

The subject matter is constituted by five horses, living in a station so inaccessible that it is not visited by touring veterinary officers, and where *Glossina morsitans* infestation is so intense that no horse had previously survived there for more than a few weeks.

In compliance with the request of the present solitary European resident in this station it was decided to make an attempt to keep a few horses alive. At the time the treatments were started, Naganol, in spite of its reputed unsuitability for repeated adminis-

tration to horses, seemed the most promising preparation to use, although treatment was undertaken more in desperation than with any reasonable hope of achieving one's object.

The dose of Naganol for horses recommended by the manufacturers is 2 to 4 gm. as a prophylactic, or a course of three doses of 2, 4 and 4 gm. at intervals of eight days for curative treatment. Dosage should vary according to weight, and the small size of the average Sudan country-bred pony would qualify him for a relatively small dose. General reports on the use of Naganol in horses have not been favourable, since, apart from its uncertain curative effect, its injection may be followed by rather alarming after-effects, such as swellings, skin eruptions, and symptoms resembling those of laminitis; and persistent administration is said to set up nephritis. However, nothing better than Naganol could be thought of, and the cases under report were treated with it.

The function of the writer was to suggest a line of treatment, to get supplies of Naganol to the owner, to give occasional advice by wireless, and to examine an occasional blood film. The owner gave all injections, entered up temperature charts, and made detailed clinical notes. This paper is thus mainly a record of his observations.

The course of treatment suggested (in all cases this was curative, and not prophylactic) was to give 2 gm. doses of Naganol intravenously at intervals which had to be determined in each case by the reaction of the patient to the drug on the one hand and to the trypanosomes on the other; if the drug affected a horse too severely the dose was to be reduced; if symptoms of trypanosomiasis (chiefly fever) recurred at short intervals the intervals between doses were to be shortened, and so on. It was also decided that if after a number of well-spaced injections no symptoms of trypanosomiasis recurred treatment might be suspended in order to see whether a cure had been temporarily effected; it was of course known that reinfection would occur sooner or later. It is now convenient to record the individual cases.

CASE NO. 1. - HORSE "DARB," JUNE, 1930.

A Sudan country-bred stallion, aged 16 years when first exposed. Developed *T. brucei*. Given 2 gm. doses of Naganol at approximately ten-day intervals for two-and-a-half years without any ill-effects beyond a small transient scurfy patch sometimes appearing on the top of the neck a day or two after an injection. Attempts to lengthen the period between doses were always followed by rise in temperature about ten or twelve days after the preceding dose. Attempts to reduce the dose to 1 gm. were similarly unsuccessful. Towards the end of 1932 the horse occasionally developed a "thick leg" which went down with exercise but reappeared with rest. This phenomenon could not be

directly associated with the injections (by now, be it noted, the horse was nearly 19 years of age). The last consignment of charts and records was received in Khartoum at the end of 1934, by which time 130 injections, nearly all of 2 gm., had been given. Early in 1936 the photograph here reproduced was received with the report that the horse was in first-class condition—a fact which is obvious from the photograph.

Although full detailed records up to the date of writing have not come to hand, it is estimated that horse "Darb" has over a period of five-and-a-half years had at least 180 injections, nearly all of 2 gm., of Naganol, following which he is in good condition and carrying a heavy man at the age of about 22 years. No blood examinations have recently been made, and in view of the treatment in progress these would hardly be informative. It is, however, fairly evident from the latest available case notes and temperature charts that he is probably not cured, or alternatively that he repeatedly becomes reinfected, since any attempt to suspend treatment for a few weeks is followed by an acute attack of fever.

CASE NO. 2. HORSE "MALAKAL," MARCH, 1931.

Sudan country-bred stallion, aged six years when first exposed. Developed *T. brucei*. Treatment adopted as in the preceding case.

This horse did not stand the Naganol injections nearly so well, and each dose was followed by swelling of the legs, prepuce or scrotum. Attempts to reduce the dose to 1 gm. had to be abandoned, since doses of this size did not cut short the febrile attacks. It was similarly found impossible to increase the interval between the 2 gm. doses, which after a while had to be given fairly regularly about every twelve days.

At the end of 1932, after 50 injections, the horse was outwardly in good condition, but was reacting as markedly to the Naganol as when treatment was first undertaken. Owing to this drug-sensitiveness the owner was unable to work him, but determined to persist with the treatment.

This state of affairs continued until the horse suddenly died in April, 1934, having had 89 doses of Naganol, mostly of 2 gm., over a period of almost exactly three years. The cause of death was not ascertained.

CASE NO. 3. HORSE "KHARTOUM," APRIL, 1931.

Sudan country-bred gelding, aged seven years when first exposed. Developed *T. congolense*. The usual treatment was undertaken.

The effect on the disease was the same as in the preceding cases: no dose of less than 2 gm. would cut short the febrile attacks, and doses had to be given at not much more than ten-day intervals.

The patient withstood the treatment only fairly well at the outset, each injection being followed by swelling of the prepuce and belly, and usually by an eczematous eruption around the anus. In consequence of the latter symptom the anus became cracked and permanently thickened, but this did not seem to inconvenience the horse. Occasionally, at irregular times, colicky pains developed, but it was not possible to associate these definitely with the injections. After about 50 injections swellings and eruptions ceased to develop, but irregular colic continued. Finally, after the treatment had continued for a little over two years, the horse developed an attack of such violent abdominal pain that the owner resorted to destruction on humanitarian grounds. It is to be noted that this attack occurred eight days after the last injection. The number of injections given is not exactly known, as a few of the earlier charts were mislaid, but the total lay somewhere between 65 and 70.

CASE No. 4. APRIL, 1934.

Abyssinian pony, no particulars. Developed *T. brucei*. Treatment along the prescribed lines was carried out for nine months. For the first five months an average of one injection every three weeks was sufficient to keep the disease under control, but it was regularly noted that if less than 2 gm. were given fever supervened a few days later and another dose had to be given. From the sixth month onwards the infection intensified so that injections at intervals of five or six days were insufficient.

In the early stages, when injections were well spaced out, the pony withstood the treatment fairly well, but later on, when frequent repetition became necessary, he developed swellings under the body and at all extremities, and symptoms as of sub-acute laminitis appeared. At the end of nine months' treatment, during which 22 injections were given, the owner decided on destruction.

CASE No. 5. NOVEMBER, 1934.

Sudan country-bred pony, *T. brucei*, usual treatment started.

The first dose had most startling effects, causing almost immediate urticarial swellings, colic, and a violent desire to bite the legs. Subsequent doses had similar effects and, apart from this, had no effect on the febrile attacks, which were presumably due to the trypanosome infection. A dose of 4 gm. was tried about two months from the outset, and this produced symptoms reminiscent of general laminitis. After four months of treatment the horse went off feed, developed large swellings under the chest and belly and around the mouth, and became partially paralysed. The owner decided on destruction. In all this horse received 19 injections over a period of four months.

DISCUSSION.

The substance of this paper is perhaps rather of academic interest than of practical importance. Nevertheless, circumstances are conceivable in which the possibility of keeping horses alive and moderately workable in tsetse areas, if only for limited periods, might have to be seriously considered.

It is to be noted that four of the five horses were recorded as having *T. brucei* and the fifth *T. congolense*. It is, however, highly probable that all five harboured both species of trypanosome, since both abound in the district, although in horses and mules *T. brucei* is usually the first one to make its appearance in cases of double infection.

The question of cure or otherwise has therefore to be considered as though double infections were at issue. Owing to the impossibility of withdrawing the patients from the infected zone this question is, however, of secondary importance. Actually, the rapid recurrence of fever following attempts to suspend treatment indicates that cures were not effected, and that the treatment did no more than keep the infection in check. The point at issue, therefore, is whether any particular horse can withstand the quantity of Naganol necessary to achieve this object, and this factor will in turn be influenced by the severity of the infection and the intensity of the treatment necessary to control it.

The first conclusion, which is confirmed by all the foregoing cases, is that no dose of Naganol of less than 2 gm. is of any use, and in horses which persistently react severely to this dose there is no point in continuing treatment; the horse may be kept alive for some months, but will be unworkable. Such a case was No. 5.

The next important observation is that, in horses which withstand well spaced out doses, the necessity for shortening the interval in virtue of increasing virulence of trypanosome infection may lead to development of toxic symptoms. Such a case was No. 4, which withstood injections at three-weekly intervals for five months, but failed to withstand the more closely crowded doses which later became necessary.

It appears that some horses, although not withstanding the injections very well in the early stages, may with passing time develop a rather greater tolerance to the drug. Such a case was No. 3, which gradually developed a tolerance in a period of a year or more. It is not certain whether this horse eventually succumbed to the effects of Naganol or not. It suffered from periodic "colic," but the attacks could not be referred to Naganol injections, and the severe attack during which it was destroyed occurred eight days after the immediately preceding injection. The horse in any case survived for two years and was workable for most of this period. Case No. 2, on the other hand, responded badly throughout, and although it survived for exactly three years it was as a rule unworkable.

The only unqualified success was Case No. 1, which has now tolerated Naganol treatment for nearly five years and has had nearly 200 injections. In his case the essential factors appear to operate in the right proportions; he can withstand 2 gm. of Naganol at reasonable intervals, and the trypanosome infection can be controlled by the doses he can withstand.

A summary of results of the five foregoing cases is thus:—

Case No. 1 received nearly 200 injections and remained workable and in good condition—and is still so—over a period of nearly five years.

Case No. 2 remained in outwardly good condition for three years, but only remained workable for about half this period.

Case No. 3 withstood treatment only fairly well, but was workable for nearly two years.

Case No. 4 withstood treatment perfectly for five months, but when more intensive treatment became necessary he could not withstand it.

Case No. 5 could not withstand the treatment from the outset, quite apart from the intervals between doses.

The conclusion therefore is that most horses can tolerate Naganol to a far greater extent than is commonly believed, and, in spite of undesirable reaction to the drug in many cases, they can be maintained in workable condition for some months at least. No dose of Naganol lower than 2 gm. will check a trypanosomiasis relapse, and the outcome in a horse which is not inherently intolerant of the drug will depend on whether or not the trypanosome infection becomes so severe, in the sense that relapses occur at short intervals, that an intolerable intensity of treatment becomes necessary.

It would thus be possible to maintain the majority of horses in workable condition in a tsetse area for a few months by giving 2 gm. doses of Naganol every ten or twelve days. The procedure would be expensive and laborious, and would only be justifiable in very exceptional circumstances.

In conclusion, it is necessary to acknowledge the efforts of the owner of the horses, Captain J. K. Maurice, M.B.E., M.C., Sudan Political Service, who not only carried out the drug treatment, but in addition kept case records with a detailed accuracy that inspired the writer to publish this short note.

PRIMARY IRIDO-CYCLITIS IN FOWLS:
A CONDITION DISTINCT FROM THE EYE LESIONS
OCCURRING IN NEURO-LYMPHOMATOSIS.

By H. P. BAYON,

*Molteno Institute for Research in Parasitology,
University, Cambridge.*

IN the course of fowl-paralysis investigations during these last three years, I have frequently observed a peculiar morbid condition of the eyes in fowls, which, though very similar to the irido-cyclitis occurring in connection with neuro-lymphomatosis and to which I referred in a previous paper in the *Veterinary Record* of April 23rd, 1932, yet differed in its incidence and course, so that it can be considered a distinct disease and deserves to be separately investigated.

Particular attention has been given to the eyes of poultry in general, because the opinion is commonly held that grey or "pearly eyes" which usually designate a pale iris and pupils which do not react to light, are *always* a sign of neuro-lymphomatosis.

I have, however, noticed on farms that eye lesions may occur quite independently of neuro-lymphomatosis and in numbers sufficient to call attention to the economic importance of the ailment.

In these examinations I must acknowledge the kind help of Miss Marjorie Perrers Taylor, M.R.C.S., who has repeatedly examined the eyes of living birds with the slit-lamp microscope, diagnosed the conditions and performed iridectomy on fowls suffering from secondary glaucoma following irido-cyclitis.

(1) SYMPTOMATIC EYE LESIONS IN NEURO-LYMPHOMATOSIS.

In one of the first papers on the subject of fowl-paralysis, L. P. Doyle (1926) mentions the occurrence of eye lesions in some of the birds and concludes: "An essential relation between the peculiar iritis and the more strictly nervous affection has not been proved. However, the frequent association of the two conditions in affected flocks and the similarity of the cellular infiltration tempt one to assume that the two are aetiologicaly identical."

In their classical paper on fowl-paralysis, Pappenheimer, Dunn and Cone (1929) mention that partial or complete blindness developed in three birds out of 60 with spontaneous fowl-paralysis; in these during life a change in the colour of the iris was noticed which turned from yellow-brown to a bluish-grey. After death the iris was found thickened, oedematous and infiltrated with wandering cells of two distinct types: small, deeply-staining lymphoid cells with little cytoplasm and larger mononuclear with an eccentric

nucleus and strongly basophilic cytoplasm (plasma cells?). These larger elements were aggregated into compact clusters, situated especially near the anterior surface. Occasionally eosinophilic polymorphonuclears were also present, together with slight oedema and granular coagulum and in the anterior chamber in some sections mononuclear cells of various types were detected. Cornea normal, retina unaltered and optic nerve slightly infiltrated. Groups of lymphoidal cells occurred in conjunctiva and extra-ocular muscles.

Most authors in recording their observations on fowl-paralysis mention eye lesions in some of the ailing birds; thus, in this country, McGaughey (1930), Warrack and Dalling (1932), Bayon (1932); but the first exhaustive paper on the subject is that of Findlay and Wright (1933), who consider what they call "epidemic blindness" in fowls as closely related to fowl-paralysis. The lesions were found in the optic tracts and nerves, choroid, retina and pecten, iris, intrinsic and extrinsic ocular muscles as well as in the conjunctiva and connected tissue of the orbit and consisted of infiltrations with lymphoidal cells. No evidence of ocular lesions similar to those due to lack of vitamin A could be detected in these birds.

Moreover, Jaensch and Lerche (1934) have histologically examined 37 eyes of 22 paralysed fowls and observed inflammatory reactions in the uvea, usually in both eyes of individual birds. In the iris they found cellular aggregates around the superficial vessels, which then progressed so as to form massive infiltrations that replaced the muscular fibres. In the radiary region the inflammatory reactions were milder, consisting in round cell infiltrates which supplanted the unpigmented epithelial layer. The walls of Schlemm's canal were thickened by cellular deposits, so that they might even swell into the lumen. In the vascular area there were dilated vessels or acute inflammatory reactions, similar to those seen in lymphatic leukæmia in man. The retina might be filled by lymphocytes, histiocytes and pigment cells. Similar round-cell infiltrations were noticeable in the optic nerve and chiasma. The authors concluded that the visual disturbances noticeable in fowl-paralysis were due to an inflammatory process in the paths of vision due to uveitis and its complications, accompanying trophic disturbances and lesions of the choroideal tunic, possibly caused by toxic action.

Hartwick (1934) described irido-cyclitis as a special form of "Marek's paralysis" and added that such birds were particularly dangerous since they lived long, scattered the "virus" in their droppings and continued to lay eggs. These views do not seem to agree with the general opinion in regard to neuro-lymphomatosis, which is not easily transmissible, even by inoculation, and in which disease either egg-laying does not occur or stops once the ailment is evident.

Oakley (1935) in a paper on "Lymphomatosis" referred specifically to an ocular type of "fowl-paralysis" and stated that the ocular lesions should be separated from irido-cyclitis and certain congenital deformities, like colobomata or defects in the iris. In irido-cyclitis there is bulging of the anterior chamber, with hazing of its contents, followed by fixation of the iris to the lens, irregular distortion of the pupil, usually associated with fibrotic changes in the iris and marked loss of pigment.

In the ocular lesions of fowl-paralysis the infiltration of the iris and choroid consisted nearly entirely of cells of the lymphoid series, embedded in a very scanty stroma. Oakley (1935) further remarked that the lymphomatous infiltration of the iris might give rise to secondary attacks of irido-cyclitis, which might engraft on the original process, which need not be directly responsible for such changes (p. 1001).

It seems therefore that this author considered irido-cyclitis closely allied with lymphomatosis, which, in his opinion, could precede the eye lesions.

(2) IRIDO-CYCLITIS AS A DISTINCT AILMENT.

The only reference I have found in literature relating to eye lesions in fowls, apart from paralysis, is that of Veenendaal (1929), who stated that he had observed a sporadically occurring irido-cyclo-choroiditis in several birds at the same time; this regularly ended in blindness, though the fowls did not appear particularly ill. In the beginning there was photophobia, watery eyes and more or less evident cloudiness of the cornea. At the same time newly-formed vessels appeared at the edge of the sclera, the iris became paler and swollen; at times the humor aqueus was clouded. Later the iris became atrophic and adherent to the lens, so that cataract resulted. Inoculations did not succeed.

McGaughey and Downey (1930) in describing the pathology of ten birds with fowl-paralysis, mention that in the eyes with iritis, the cellular infiltration was polynuclear and eosinophilic, therefore differing from the lymphoid type observed in the peripheral nerves. These authors rightly conclude that the iritis they observed may not be primarily due to the same aetiological factor. It seems generally accepted that the typical eye lesions occurring in fowl-paralysis consist in a coarse infiltration of the iris by lymphoid cells, which immobilise the pupil and cause it to become distorted.

First Field Observation.

The first time that I noticed eye lesions as a distinct morbid condition was on the farm of R.H.P. (Cambs.) in August 1934. Nine hens were affected out of a batch of 200 which had been bought as three-months-old pullets; no information was available that could explain the causation of the eye lesions, which

were noticed a few weeks after purchase. No symptoms of fowl-paralysis occurred before or within two years among these birds, though it must be said that all blind birds were culled out. Five live pullets were examined; in these both eyes bulged out abnormally and were obviously painful for the birds moped and shunned the light; the iris appeared grey, blurred and the pupil small and did not react to light. These were instances of secondary glaucoma; a condition resulting from a morbidly increased tension of the tunics of the eyeball, produced by mechanical interference with the fluid circulation within the eye, following irido-cyclitis.

The birds were killed and when the anterior chamber of the eye was incised, fluid spurted out. Inner organs were healthy.

Miss M. Perrers Taylor operated on the glaucoma of two birds, which thereupon began laying; after five months of apparent good health, the hens were killed and anatomically examined; no trace of neuro-lymphomatosis.

The operation consisted in iridectomy; a segment of the iris was drawn through an incision at the limbus and excised, thereby affording a communication between the anterior chamber and the sub-conjunctival tissues so that a regular flow of the fluid could take place, thus relieving the pressure and consequent pain. The iris was noticeably friable and easily torn; healing took place in every instance without complications. Ether anaesthesia was employed.

Second Field Observation.

The next instance of blindness and "pearly eyes" occurring in numerous chickens about the same time was observed on a farm (W.M.) in Essex, which I visited on repeated occasions beginning in 1933.

Here "colds" were originally the prevailing ailment; numerous White Wyandotte pullets could be noticed with moist nostrils, or breathing with a slight rattle in the throat or showing swollen sinuses. After *post-mortem* examination the bones were noticeably soft, ill-developed and all inner organs pale; trachea slimy and pale, not injected. It seemed as if there might be some disturbance in the calcium metabolism and that the spread of the "colds" may have been due to some error in brooding and feeding during the early stages. In any case certain alterations in method and feed formula were recommended and the new appearance of colds ceased. Soon after, single chickens developed "pearly eyes" which were accompanied by blindness, so that the birds could not pick up food on range and suffered in health. Next season the colds did not reappear, but blind birds became much more numerous. A dozen blind birds were picked out, killed and anatomically examined; this time the bones were firm and well developed, but chronic coccidiosis was evident in the bowels of seven chickens. In the course of the season the number of blind birds increased, till in my estimation it reached 200 to 300,

though according to the farmer double the number would be nearer the mark. Further *post-mortem* examinations revealed the "pearly eye" to be the single lesion in the birds examined, with the exception of a lymphoma of the ovary of one.

Energetic culling and certain additions to the feed were recommended, the ultimate result of which remains still to be seen. It is not denied that single instances of paralysed birds or hens with a dropped wing were detected at intervals, but these did not occur in a higher proportion than about one paralysed bird to 30 with blindness.

Third Field Observation.

A further occurrence of this peculiar condition was on a farm in Scotland. This farm was personally visited to make sure that fowl-paralysis was not present in any of the other birds; here also "colds" were first noticeable, then the subsequent season (1935) blindness and eye lesions occurred in a large proportion, possibly 20 or 30 per cent. out of 300 layers. The farmer sent me three live hens for observation and on arrival in Cambridge the condition of the eyes was as follows:—

No. 1.—Active irido-cyclitis in one eye; the other normal except for paleness, due to lack of pigmentation.

No. 2.—Irido-cyclitis in both eyes; in one eye the pupil is contracted to pin-head size and in the other the pupil is completely bound down to the lens, which is opaque.

No. 3.—Conjunctivitis in one eye.

The hens were placed on free range, where Nos. 1 and 3 came into lay; normal full-sized eggs; both ceased laying about November and now, end of December, the condition is: In No. 1 the condition of the eye remained stationary; in No. 2 the eyesight had apparently become worse, the bird moped and appeared unable or unwilling to find its food; therefore placed in a single pen and both eyes operated upon; appetite thereupon was regained. In No 3 the disease progressed gradually till glaucoma developed in December, which, however, does not seem to have interfered with the health or activity of the bird.

In addition to these field observations, six White Wyandotte hens with early stages of irido-cyclitis were obtained from various sources; the birds were placed on range and when blindness developed were housed in single pens. All these hens came into lay and though observed during nine months did not become paralysed and when killed no lesions of the peripheral nerves or inner organs could be detected.

(3) PERSONAL OBSERVATIONS ON THE HISTOLOGY OF IRIDO-CYCLITIS.

It is generally admitted that the typical lesion of the eye in fowl-paralysis consists in infiltration of the iris muscles by

lymphoidal cells which immobilise the pupil and cause it to become distorted.

Apart from this, the following iris changes may occur in fowl-paralysis but also as a separate and independent ailment:—

(1) Cellular exudate, particularly in the anterior chamber; the iris converted into a pale smooth tissue with many dilated vessels. The lens capsule covered by a white membrane; clumps of pigment noticeable on one side. This is the acute stage.

(2) The pupil is adherent; the iris becomes atrophic and has lost its pigment through the effects of some inflammatory process. As the result of the lack of pigment the capillaries appear dilated and are more sharply defined than usual. The crystalline is clear.

(3) Anterior chamber cloudy, iris firmly adherent, bound down; no pigment, capillaries clearly visible, no light reaction. Eye hard, apparently painful; excess of fluid in anterior chamber; secondary glaucoma. This is a chronic condition.

These alterations can be detected in the living bird by means of the slit-lamp eye microscope.

The three stages correspond to definite histological pictures, which were observed in sections of eyes obtained after killing the fowls, the specimens being fixed in Zenker's fluid; celloidin sections were kindly made by Mr. Dick of the Pathological Laboratory of Moorfields Eye Hospital, London.

What may be considered the early or incipient stage was distinguished by a definite dilatation of the blood vessels of the vascular area below the retina; similar congestion occurred in the vessels between the fibres of the iris muscles.

The optic nerves were discretely infiltrated by rows of small lymphoidal cells, but it may be noted that these cells occurred in interrupted single files and not the massive grouping so often seen in fowl-paralysis.

In the second stage the process of congestion progressed to early inflammatory changes, remarkable through the accumulation of lymphoidal cells in the neighbourhood of the dilated blood vessels; the grouping of these cells was irregular and patchy. The pigment (the absence of which was noticeable in the living eye) was found clogging the lymphatics in the shape of small clumps.

The third or glaucomatous stage was heralded by an intensification of these processes; massive lymphoidal cell aggregates invaded the iris, compressed the muscles, obliterated the pigment, clumps of which could be found in the optic nerve. The lymphoidal infiltration of the optic nerve had not progressed, but either remained stationary or gone over to atrophic changes. The lumen of Schlemm's canal was much reduced by a jagged swelling of the walls caused by lymphoidal aggregates.

On two occasions we were able to obtain from a fowl a diseased eye, whilst the corresponding one was seemingly healthy and

unaltered. On microscopical examination it was, however, noticeable that the seemingly sound eye was also affected by alterations in the initial stages, which were only slightly progressive.

It is quite evident that on anatomical grounds alone it is scarcely possible to distinguish between the lesions of irido-cyclitis occurring as an autochthonous eye ailment and the similar condition seen in conjunction with neuro-lymphomatosis. Nevertheless, the course of the simple morbid eye condition is so different from that of the generalised lesions of the peripheral nerves, that unless it is admitted that fowl-paralysis occurs without paralysis and without the anatomical changes that characterise the condition known as "neuro-lymphomatosis," it seems more logical to separate the diseases than to unite them.

(4) WHY IRIDO-CYCLITIS IS CONSIDERED TO BE A SEPARATE EYE DISEASE IN FOWLS.

Findlay and Wright (1933) in their paper on eye lesions in fowl-paralysis state very fairly why the suggestion has been made by other authors that "epidemic blindness" is not due to the same agent as fowl-paralysis; this, they say, is largely on the following evidence:—

(1) The number of fowls exhibiting signs of blindness varies greatly in different outbreaks of fowl-paralysis.

(2) In certain epidemics blindness has been unaccompanied clinically by any other signs of fowl-paralysis.

(3) The lesions in the eyes differ from the lesions of fowl-paralysis in containing numbers of eosinophile polymorphonuclear leucocytes.

The first two of these arguments do not weigh with the authors because they are not convinced that where there is irido-cyclitis (a more correct term, for many fowls with the condition are not blind) a careful histological study of the brain, cord and peripheral nerves will not yield evidence either of perivascular infiltration of the central nervous system or of accumulation of lymphoidal cells in the nerves; these, they believe, would prove the presence of neuro-lymphomatosis. They do not consider the presence or absence of polymorphonucleated cells of sufficient weight on which to base a difference in ætiology.

Stated in this comprehensive and moderate manner it is not possible to deny or support such views in a few words. In my opinion it is quite immaterial whether few or many instances of peculiar types occur in the course of an epidemic, as long as the anatomical lesions can be correlated; hæmorrhagic smallpox is rare in epidemics, but its causation is assured. Even if irido-cyclitis were commoner in flocks where fowl-paralysis is occurring than it is in reality, the argument would not be conclusive.

In my opinion the second objection can be more easily upheld and it may be said straightaway that the majority of instances of

irido-cyclitis have been seen in flocks where fowl-paralysis was present or became apparent soon after; this in conformity with the observations of Findlay and Wright (1933). But I have been able to observe a dozen or more blind fowls on each of three farms where fowl-paralysis could not be detected at the time or certainly occurred only in single birds.

The whole question resolves itself into one of definition of terms. I consider neuro-lymphomatosis to be a disease diagnosable by the presence of paralysed limbs, anatomically caused by lymphoidal infiltration of the peripheral nerves and accompanied by increased lymphoidal aggregates in the liver, spleen and bone-marrow. Others hold that lymphoidal aggregates, irrespective of where they occur, are signs of lymphomatosis and that fowl-paralysis is a peculiar type of the widespread disease, lymphomatosis.

If irido-cyclitis is a localised manifestation of a generalised disease, which is known to show a high mortality, then it is difficult to understand why in many instances the fowls affected after a brief period of ill-health seem to recuperate completely except, of course, in relation to the sight disability, go on laying and even when observed for a year or more do not become paralysed. If irido-cyclitis were transmissible by inoculation, knowledge might be obtained by experiment; as it is, field observations have been made to yield some information regarding the relative occurrence of the fowl-paralysis and irido-cyclitis and to show that the two diseases do not always run parallel.

Is Irido-Cyclitis Connected with Heredity?

Because fowl-paralysis will appear in successive seasons in some definite strain and also when it first makes its appearance on a farm it is usually connected with birds from some particular source, it has been suggested that the ailment is transmitted through the egg or heredity. Some geneticists will not agree with this view because the transmission is too fitful and irregular; though it must be admitted that in human pathology similar capricious parental transmissions have been accurately observed over several generations, *e.g.*, in relation to some obscure ailments or anatomical peculiarities, like pseudo-hæmophilia or even the condition known as "Hæmorrhagic multiple telangiectasis." Osler (1901) and others later on.

In any case the careful field investigations of Marginson and McGaughey (1931), Asmundson and Biely (1932) and the experimental investigations of Biely, Palmer and Asmundson (1932) can be quoted as evidence bearing on the strain-linkage of neuro-lymphomatosis in fowls.

In relation to irido-cyclitis, its presence in closely-related stock on a farm need not necessarily be interpreted as due to some hereditary factor; several other explanations can be suggested and

a choice among these can be made when the ætiology of the disease is more firmly established.

Causation of Irido-Cyclitis.

The ætiology of the condition in fowls is still obscure; in man the disease is at times a complication of gonorrhœa, but then as the result of toxic action, for the gonococcus is not found in diseased iris. Similarly, in man, irido-cyclitis may occur as the result of tubercular-toxic action in the course of lung tuberculosis. Irido-cyclitis as a complication of tropical spirochaetosis (tick fever transmitted by *Ornithodoros moubata*) is well known.

In fowls, particular attention has been given to the presence of intestinal parasites, like coccidia or tapeworms; these did not occur with sufficient regularity to warrant the suggestion that there was a definite connection between the parasites and the condition in the eyes. Avian tuberculosis was present in one fowl, in which discrete lesions of the bowel were detected.

On two farms the frequent occurrence of eye lesions was heralded by the appearance of "colds" the previous season. In the first instance observed a deficiency in the diet could not be definitely ascertained; in the third, however (Scotland), the mash fed was definitely low in vitamin A and indeed a couple of birds were found with definite and clear nutritional roup, recognisable through the condition of the gullet and the swollen eye sinuses. Moreover, the addition of maize and alfalfa meal together with cod-liver oil prevented a repetition of the eye lesions in this year's stock. Eyes that were already affected did not improve as the result of the alteration of the feed.

Against these indications one must remember that the lesions seen in these eyes differ from those seen in xerophthalmia through acute avitaminosis A.

Treatment.

Irido-cyclitis and its after-effect, glaucoma, are usually noticed when far advanced; but even if recognised in the early stages the best advice that can be given is to isolate the affected chickens in quiet surroundings. Atropine, which is used in similar ailments in man, is without effect on the iris of birds. The operation for glaucoma requires surgical skill, particularly as the sclera of the fowls is abnormally hard and the iris small and friable.

When dealing with large numbers of otherwise valuable hens I have advised to place blind birds in single laying pens, where they could pick up food, which should be nourishing and well balanced in vitamins. However, if the bird is in pain the most humane thing to do is to kill it.

CONCLUSION.

The occurrence of outbreaks of irido-cyclitis, involving relatively large numbers of fowls, without the appearance of paralysis before, during, or after the development of the eye lesions, seems to suggest that irido-cyclitis is not always due to neuro-lymphomatosis, but may occur as a separate disease.

Such birds with primary irido-cyclitis, when placed in single pen cages where they have easy access to food and drink, notwithstanding their blindness, will go on laying throughout the season; whilst fowls with neuro-lymphomatosis either do not come into lay at all or cease laying the moment the disease is manifest.

Though it is admitted that there is no clear-cut difference between the anatomical lesions in the eyes of birds with primary irido-cyclitis, when compared with those detectable in the eye affection occurring in the course of fowl-paralysis, yet the different occurrence and course of the two ailments, may be allowed to suggest a distinction in pathology, between the localised affection of the eye, when occurring alone and the generalised lymphomatosis seen in fowl paralysis.

From the standpoint of veterinary medicine it is advisable to treat cautiously the presence of "pearly eyes" in breeding birds, since it is not always possible to distinguish in the living, on the spot, certain harmless varieties of "pale eyes" from early and yet dangerous pathological lesions.

These investigations would not have been possible without the help afforded during the tenure of a Henry George Plimmer Fellowship in Pathology and repeated grants from the Agricultural Research Council to the Molteno Institute; I am also particularly grateful to the Head of the Institute, Professor David Keilin, F.R.S., for much wholehearted incentive to these researches and advice in relation to the subject.

THE ISOLATION OF *BRUCELLA ABORTUS* FROM THE MILK OF COWS WITH NEGATIVE BLOOD REACTIONS TO THE AGGLUTINATION TEST.

By T. M. DOYLE,
Veterinary Laboratory, Ministry of Agriculture, Weybridge,
and FREDERICK BECKETT,
Blandford, Dorset.

THE literature on bovine contagious abortion contains a considerable number of references to the isolation of *Br. abortus* from the milk of cows whose blood serum did not contain specific agglutinins in the amounts usually accepted as indicating infection.

Hayes and Barger (1923) recorded the case of a cow whose serum gave a negative agglutination reaction with *Br. abortus*, but which excreted the organism in the milk.

Hart and Traum (1925) state that "a correlation of the agglutination tests of the animals with the definite periods when *Bacterium abortus* was known to have been eliminated shows that this organism may be discharged from the body without its presence being suspected from the agglutination titre of the blood."

Carpenter (1926) isolated *Br. abortus* from the milk of three cows, previously inoculated with live vaccine, which had given negative blood reactions in a dilution of 1 : 60.

Carpenter and Parshall (1927) examined by guinea-pig inoculation the milk of 18 non-reactor cows in an infected herd for the presence of *Br. abortus*. A blood titre of 1 : 60 was accepted as indicating infection. Two guinea-pigs were inoculated with each sample of milk and all gave negative results.

It is stated in a footnote that :—

"One guinea-pig showed suspicious lesions of abortus infection in spleen and liver. An abortus-like culture was isolated from the spleen, but when placed with an anti-abortus serum with high-titre agglutination occurred only in the 1 : 60 dilution."

It is not clear from this evidence that the organism isolated was not *Br. abortus*.

King (1928) recorded the case of a cow in an infected herd which was excreting *Br. abortus* in the milk, but whose blood failed to react to the agglutination test. No details are given regarding the test.

Graham and Thorp (1930) carried out comparative tests with blood and milk whey in an infected herd for the presence of brucella agglutinins. The tests were made in dilutions of 1 : 50 and 1 : 200. A positive reaction in the 1 : 200 dilution was

accepted as indicating infection, while a positive reaction in the 1 : 50 dilution and a negative reaction in the 1 : 200 dilution were regarded as doubtful.

One animal out of a total of 172 tested showed a positive agglutination reaction with whey and a negative result with blood.

Laurens (1931) found that of 33 cows excreting *Br. abortus* in the milk six (18 per cent.) gave negative reactions with the blood. No details are given of the blood titres or of the antigen used.

Tullberg (1931) inoculated separately the milk from each quarter of the udder of 54 cows into guinea-pigs. Agglutination tests of the cows' blood were carried out at the same time.

He found that when the blood titre was 1 : 100 or higher, *Br. abortus* was present in the milk of more than 50 per cent. of the animals; when the blood titre was 1 : 70 the organism was present in the milk of 19 per cent., and when the blood titre was 1 : 10 to 1 : 20, 3 per cent. of the udders were infected.

Montgomerie and Rowlands (1932) examined 615 samples of blood sera and milk whey for the presence of brucella agglutinins. Fourteen blood samples gave negative reactions while the corresponding whey samples gave positive reactions.

An examination of the histories, breeding records and agglutination reactions obtained in previous and subsequent blood tests suggested that in twelve of these animals the results of the blood tests were correct and that in the remaining two the whey test was correct.

Caldwell, Parker and Medlar (1934) isolated *Br. abortus* from the milk of a cow, previously inoculated with "live" abortion vaccine, whose blood failed to react to the agglutination test. No details are given as to the agglutination titre accepted as indicating infection.

They state that the absence of agglutinins for *Br. abortus* in the blood serum of a cow in an infected herd cannot always be accepted as proof of the absence of the organism in the milk.

In their experience the presence of agglutinins in the whey is more reliable as an indication of udder infection than in the blood serum. Viriden (1934) found *Br. abortus* in the milk of one cow which had a blood titre varying from 1 : 20 to 1 : 30 over a period of two months.

Hayes and Barger (1935) studied a *Br. abortus*-infected herd for a number of years to determine the correlation between agglutinins in the blood and in the milk whey and the relationship between the agglutinins and the presence of *Br. abortus* in the milk. In carrying out the agglutination test the lowest dilution used was 1 : 50.

Br. abortus was not isolated from composite milk samples from the udder of any cow with persistently negative blood reactions.

They state: "However, in the group of 38 animals in whose udder *Br. abortus* was found one or more times, a

significant fact developed in relation to blood agglutinins and udder infection: eight, or over 21 per cent., of the cows shed *Br. abortus* from the udder either before agglutinins appeared in the blood or at a time when the blood agglutinins had receded to negative or to positive at a dilution of only 1:50."

In cow No. 342 *Br. abortus* was isolated from the milk 273 days, and again at 149 days before the blood was positive at 1:50.

In cow No. 380 *Br. abortus* was isolated from the milk on two occasions at an interval of three months when the blood was negative at 1:50.

In cow No. 349 *Br. abortus* was isolated from the udder 67 days before the test revealed the presence of agglutinins in the blood.

They conclude that: "Cows in an infected herd may have *Br. abortus* in their udders for a considerable period before agglutinins appear in the blood stream. Udders of cows having a positive blood history may continue to carry *Brucella abortus* when the blood titre has declined to negative in a dilution of 1:50.

"Cows with declining and low fluctuating blood titres may be spreaders at normal calvings when the titre is as low as negative in 1:50."

Henry, Haring and Traum (1935) carried out monthly blood and milk examinations of 20 cows in an infected herd. *Br. abortus* was not isolated from any cow with a blood titre below 1:50; as four animals only came into this category the number is too small to permit of any conclusion. They isolated *Br. abortus* from the milk of one or more quarters in 27.3 per cent. of 22 udder tests of five cows with a blood titre of 1:50.

They concluded that cows whose blood serum showed any agglutinins in a titre of 1:100 are actual or potential shedders of *Br. abortus* in the milk; and that cows with a blood titre of 1:50 may shed the organism in the milk.

The object of the work here recorded was to examine a considerable number of milk samples from non-reacting cows in infected herds, accepting a blood titre of 1:25 as indicating infection, to ascertain whether *Br. abortus* is excreted in the milk under these conditions. The tests were carried out in commercial dairy herds maintained under average farm conditions.

Technique.

Blood and composite milk samples were taken at the same time from all cows under examination and as a preliminary step agglutination tests of the blood sera were carried out.

If the blood tests revealed a considerable degree of brucella infection the milk sample from each non-reactor cow was centrifuged and the cream and deposit inoculated intramuscularly

(2 c.c.) into two guinea-pigs, and an agglutination test was made of the milk whey.

The guinea-pigs were killed about the forty-fourth day after inoculation and cultures made from each spleen on liver agar and the tubes incubated for ten days under 10 per cent. CO₂ at 37° C. An agglutination test was made of the blood serum of each guinea-pig.

The agglutination tests of the bovine and guinea-pig sera and of the milk whey were made in dilutions of approximately 1 : 25, 1 : 50, 1 : 100 and 1 : 200; in a considerable proportion of tests of the bovine sera a 1 : 10 dilution was also included.

The antigen consisted of a 72-hour-old liver broth culture of *Br. abortus*, the density of which was increased by the addition of a small amount of a 48-hour-old washed liver agar growth in order to match its opacity with Brown's No. 1 Tube. The antigen was made from a "smooth," non-thermo-agglutinable strain of *Br. abortus*.

The tests were made in small tubes, 50 mm. × 10 mm., undiluted serum being placed in a series of four tubes in quantities of 0.04, 0.02, 0.01 and 0.005 c.c. respectively and 1 c.c. of antigen added to each tube with an automatic distributor.

Positive and negative serum controls and an antigen control with carbolised saline were included with each group of tests.

The milk whey was prepared by centrifuging 40 c.c. of milk at 3,000 r.p.m. for 20 minutes. The middle milk was pipetted off, 1 c.c. of rennet added to it, and the mixture incubated for two hours at 37° C. At the end of this time a water-clear whey was present over the clot.

Isolation of Br. abortus.

A total of 309 milk samples, obtained from 17 different herds, were tested by guinea-pig inoculation for the presence of *Br. abortus* (Table I).

The organism was isolated from two of the milk samples (Herd No. 7), both of which were obtained from cows with negative blood titres in dilutions of from 1 : 25 to 1 : 200.

The organism was isolated also from the milk of one cow (Herd No. 4) whose blood serum gave a positive reaction in a dilution of 1 : 25; this was the only milk sample examined from a cow showing a positive blood titre.

One sample of milk (Herd No. 8) had a whey titre of 1 : 100, but *Br. abortus* was not isolated from the inoculated guinea-pigs. In view of the high whey titre in this instance, it would appear reasonable to believe that had further samples of milk been examined the organism would have been isolated.

Two guinea-pigs inoculated with a milk sample (Herd No. 16) had blood titres of 1 : 25 and 1 : 200 respectively (not end-titre) when examined on the forty-fourth day after inoculation, but cultures of the spleens were negative.

TABLE I
RESULTS OF AGGLUTINATION TESTS OF HERDS.

Herd Number.	Number of Blood Samples Tested.	Number Positive.	Number Negative.	Number Doubtful.	Percentage of Blood Samples Positive.	Number of Milk Samples Tested.	Number of Guinea-pigs Inoculated.	Remarks.
1	28	6	22	—	21.4	22	44	
2	23	9	14	—	39.1	12	24	
3	40	9	29	2	22.5	26	52	
4	39	15	24	—	38.4	22	44	<i>Br. abortus</i> isolated from milk of cow with a blood titre of 1 : 25.
5	30	10	20	—	33.3	5	10	
6	39	24	15	—	61.5	10	20	
7	33	7	24	2	21.2	24	48	<i>Br. abortus</i> isolated from milk of two non-reacting cows. One milk sample had a whey titre of 1 : 100.
8	33	22	11	—	66.6	11	22	
9	45	21	24	—	46.6	23	46	
10	70	22	48	—	31.4	25	50	
11	40	14	26	—	35.0	24	48	
12	14	8	6	—	57.1	5	10	
13	78	28	50	—	35.8	11	22	
14	29	3	26	—	10.3	18	36	
15	36	11	24	1	30.5	14	28	
16	43	12	26	5	27.9	20	40	
17	64	21	41	2	32.8	37	74	

Identification of the Brucella Strains Isolated.

Both strains of *Brucella* isolated were examined as follows :—

- (1) Against a known positive *Br. abortus* serum.
- (2) For growth under aerobic conditions and under 10 per cent. CO₂.
- (3) For production of H₂S.
- (4) For growth on Huddleson's dye media.

Both strains proved to be typical CO₂-sensitive strains of *Br. abortus*.

DISCUSSION.

It is difficult to correlate the results of agglutination tests obtained by different workers because of the many factors involved, such as the wide variations in opacity of antigens, different strains of *Brucella*, the different techniques used in the test and the varying agglutination titres accepted as indicating infection. In some of the reports essential details of the tests are omitted.

In face of the evidence available it would appear that the view so long held that the milk of a cow with a negative or with a low blood titre does not contain *Br. abortus* requires reconsideration.

It is true that in some instances recorded of the isolation of the *Br. abortus* from the milk of non-reacting cows the minimum blood titre accepted as indicating infection appeared, according to our standard, to be high (*e.g.*, 1 : 50). In such cases the isolation of *Br. abortus* from the milk would appear to be more a reason for accepting a lower blood titre than a reflection on the accuracy of the test.

M'Fadyean (1928) discussed the agglutination test for the diagnosis of *Br. abortus* infection and the possibility of the existence of non-specific agglutinins in the blood of normal cows. He stated that "the results give no support to the view that the blood of bovine animals free from infection with *Br. abortus* may cause complete agglutination in 1 : 25 or even in 1 : 10."

Our experience based on the testing of non-infected herds is in agreement with this view.

In carrying out tests of infected herds it is not unusual for a small number of sera to show complete agglutination in a dilution of 1 : 10 and for some of these sera at subsequent tests to be negative; it would appear reasonable to believe that the animals from which these sera were obtained had contracted a light degree of infection and overcome it.

In other samples of sera the initial titre of 1 : 10 is followed by a gradual rise to a high titre.

Gwatkin (1932) is in agreement with the general experience that the highest percentage of infected udders is found among cows with high blood titres, but is of the opinion that animals with low blood titres may have infected udders and that the blood

titre is not helpful in determining whether or not the placenta or udder is likely to be infected.

Birch (1933) states that the blood serum of normal cows usually has an agglutination titre for *Br. abortus* of 1 : 50 or lower. Birch and Gilman (1935) regard a blood titre of 1 : 25 or 1 : 50 as normal and of no consequence even in pregnant heifers.

Gilman and Cameron (1936), applying a scheme for the eradication of contagious abortion in New York State, consider animals to be infected only when they show complete agglutination in a titre of 1 : 100.

Most British workers would consider a titre of 1 : 40 in a pregnant cow or heifer as either an indication of infection, or at least as sufficient evidence to justify regarding the animal with great suspicion, and this view is based on extensive field experience, but many competent and experienced American workers would apparently regard such a titre as of little significance.

It would be inadvisable, however, on the evidence available, to draw any conclusions as to the cause of this surprising difference in interpretation of the titres that should be accepted as signifying infection; it may be the result of a difference in antigens or it might possibly be that the relative susceptibility to *Br. abortus* infection of British and American cattle enters into the question.

The observations here recorded of the isolation of *Br. abortus* from two samples of milk out of a total of 309 (0·64 per cent.) tested from non-reacting cows are perhaps of some interest. It would suggest that the danger of infection from this source is small in cows with negative blood titres in a dilution of 1 : 25.

In connection with this enquiry blood tests were made of 684 cows belonging to 17 commercial dairy herds situated in the south of England and the average herd infection was about 35 per cent.

CONCLUSIONS.

(1) Milk samples from 309 non-reacting cows in 17 *Br. abortus*-infected herds have been examined by guinea-pig inoculation for the presence of the organism.

(2) *Br. abortus* was isolated from the milk of two cows which had negative blood titres in dilutions of from 1 : 25 to 1 : 200.

(3) Six hundred and eighty-four cows, from 17 commercial dairy herds situated in the south of England, were tested for the presence of brucella agglutinins and the average herd infection was found to be about 35 per cent.

REFERENCES.

- Birch, R. R. (1933). Rep. N. York State Vet. College, 1931-32. pp. 163-170.
Birch, R. R., and Gilman, H. L. (1935). Rep. N. York State Vet. College, pp. 67-73.
Caldwell, D. W., Parker, N. J., and Medlar, E. M. (1934). *J. Infect. Dis.*, 55, pp. 235-242.

- Carpenter, C. M. (1926). *Cornell Vet.*, Vol. **16**, pp. 133-136.
- Carpenter, C. M., and Parshall, C. J. (1927). *Ibid.*, Vol. **17**, pp. 234-235.
- Gilman, H. L., and Cameron, H. S. (1936). *Ibid.*, Vol. **26**, p. 113.
- Graham, R., and Thorp, F. (1930). *J. Infect. Dis.*, Vol. **46**, pp. 260-262.
- Gwatkin, G. (1934). *Canad. Pub. Health J.*, Vol. **25**, pp. 5-9.
- Hart, G. H., and Traum, J. (1925). Univ. California Agric. Exper. Stn. Tech. Paper No. 10.
- Hayes, F. M., and Barger, E. H. (1923). California Agric. Exp. Stn. Rpt., pp. 238-239.
- Hayes, F. M., and Barger, E. H. (1935). *Hilgardia*, Vol. **9**, pp. 527-542.
- Henry, B. S., Haring, C. M., and Traum, J. (1935). *Ibid.*, pp. 545-566.
- King, M. J. (1928). *Vet. Med.*, Vol. **23**, pp. 492-493.
- Lourens, L. F. (1931). 2nd Congrès Internat. Pathol. Comp.
- M'Fadyean, J. (1928). *J. Comp. Path. & Ther.*, Vol. **41**, pp. 362-369.
- Montgomerie, R. F., and Rowlands, W. T. (1932). *Vet. J.*, Vol. **88**, pp. 227-236.
- Tullberg, K. (1931). *Skand. Vet. Tidskr.*, pp. 389-404.
- Viriden. (1934). *Ibid.*, Vol. **24**, pp. 711-17.

PARALYSIS IN FOWLS AND SCRAPIE IN SHEEP. (A COMPARISON.)

A. BROWNLEE, B.SC., M.R.C.V.S.

WHILE the author, then engaged in research work in the laboratories of the Animal Diseases Research Association, was investigating that disease of sheep known as scrapie, " nibbling " disease, or " trotting " disease, he observed on studying literature on fowl paralysis that these two diseases had many points in common, and the object of this paper is to draw attention to this similarity by abstracting records of the observations of various workers on each disease. No attempt has been made to make a complete survey of the literature and only those characters which appear to be generally accepted as features of the respective diseases are dealt with.

Each disease is dealt with under the following heads :—

(1) BOTH DISEASES SHOW A FAIRLY WELL-DEFINED AGE OF MAXIMUM INCIDENCE.

Fowl Paralysis.

Doyle (1926). The disease occurs mainly in birds four to eight months old. However, cases do occur in birds as young as one month and in some as old as one year or more.

Jungherr (1935). The disorder attacks primarily young birds three to ten months old but has been observed at the age of five weeks.

Pappenheimer et al. (1929.) Young birds are most frequently affected. Earliest case at twelve weeks and the oldest at 15 months 18 days.

Patterson et al. (1932). The earliest age in their experience was 41 days. In general, the nerve and eye types are initiated in from four to eight months.

Seagar (1933). The age of maximum incidence is three to six months.

Thomas and Hamilton (1935). The monthly incidence is highest in the summer, when the current year's birds are coming into lay.

Scrapie.

Gaiger (1924). When about two years of age is the commonest time. Ewes of any age over two years can take the disease; plenty of instances of tups developing the disease at two years old; less common in older tups.

M'Fadyean (1918). Disease is seldom seen in animals under 18 months old. It is the rule that far more cases occur in the so-called gimmers or two-year-olds. Young rams are most liable when about two years old.

M'Gowan (1922). Cases occur in two-year-olds in great majority.

Stockman (1913). The disease is rare in animals under 18 months old. It may appear at any age after 18 months.

(II) IN BOTH DISEASES THERE IS TYPICALLY LITTLE OR NO SYSTEMIC DISTURBANCE.

Fowl Paralysis.

Pappenheimer et al. (1929). The duration of the disease is variable. With proper care life may be prolonged indefinitely and nutrition and general condition are often surprisingly good, even after months of complete helplessness, when birds are kept in confinement with access to food and water.

Patterson et al. (1932). The course is very variable and may be rapid or slow. Some cases will apparently remain in good health, except for a local paralysis, for a long time. The appetite is generally maintained throughout the course of the illness. They have observed cases that were practically helpless that would eat and drink as long as they could reach the food and water containers.

Warrack and Dalling (1932). Lamé birds as a rule have a perfectly healthy appearance, but the lameness is progressive and ultimately in days or weeks a general paralysis is found accompanied by rapid and marked emaciation. The general healthy appearance of the affected birds usually persists and if hand fed they may live for weeks. They have kept paralysed birds at the laboratory for three months.

Scrapie.

Friedberger and Fröhner (1910). Under the terms "trotting" or "nibbling" disease, the condition is described as a chronic non-febrile disease.

Gaiger (1924). Animal eats well and ruminates. Temperature is rarely above normal.

M'Gowan (1914). In the early stages the animal is in the pink of condition. Appetite is preserved to the end. Temperature remains mainly normal.

Stockman (1913). Duration three to four months. Animal feeds as usual and ruminates.

(III) BOTH DISEASES ARE INVARIABLY OR ALMOST INVARIABLY FATAL.

Fowl Paralysis.

Pappenheimer et al. (1929). The outcome is usually fatal, but spontaneous recovery may rarely occur.

Patterson et al. (1932). Death is the outcome in practically all cases, though respites and arrested progress are sometimes noticed.

Scrapie.

Gaiger (1924). No record of recovery.

M'Gowan (1922). Almost invariably fatal; three cases of recovery are recorded.

Stockman (1913). Scrapie is looked upon as an affection which always ends fatally.

(IV) IN THE CASE OF BOTH DISEASES THE CAUSE IS UNKNOWN.

Under this heading it would seem sufficient to state that, although several ætiological agents have been incriminated, in neither disease has the evidence submitted been sufficiently conclusive to be generally accepted by other investigators.

It may be of interest to point out here that in both diseases parasites, which commonly infest their respective hosts (fowl and sheep) and which normally do not cause any symptoms at all resembling those of the diseases under discussion, have been incriminated as ætiological factors.

In fowl paralysis coccidia and intestinal worms have been associated with the ætiology of the condition by various workers [*vide* "A Review of the Literature," *Biely and Palmer* (1932)].

In scrapie *Sarcocystis tenella* has been incriminated by *M'Gowan* (1914).

(V) BOTH DISEASES HAVE BEEN ASSOCIATED WITH HIGH FEEDING.

Fowl Paralysis.

Blount (1932). It is the modern tendency to use forcing mashers which grow big chicks in a short time. It is not improbable that the more recent manifestations of fowl paralysis are in the main due to nutritional factors which have stimulated the primary causal factor on a widespread scale.

Patterson et al. (1932). Their observations indicate that the disease is not due to any dietetic deficiency. In fact there is some evidence to indicate that birds in a poorly-nourished state from whatever cause are somewhat less susceptible than are those that are well nourished.

Seagar (1933). Artificially forced and pampered flocks are most liable.

Scrapie.

Friedberger and Fröhner (1910). Pampering the young and unhealthy forcing are given among predisposing causes.

Gaiger (1924). It is the view of many that high feeding helps to bring out the disease which might otherwise have remained latent.

M'Gowan (1922). In the earlier records it was observed that high feeding brought out the disease and that sheep on rich meadows were more liable to the disease than sheep pastured on poor soil.

Stockman (1913). He quotes an experienced shepherd, who said, "The better the food the worse they become."

(VI) BOTH DISEASES HAVE BEEN PREVALENT IN STOCKS IMPROVED BY SELECTIVE BREEDING.

Fowl Paralysis.

Though the author has not found specific mention of this possible aetiological factor, it is universally recognised that recently there has been a marked grading up of many breeds of poultry with a view to high egg production.

Scrapie.

Friedberger and Fröhner (1910). The disease is common in the very fine Electoral flocks of Germany, rarer in the commoner sheep of the country or the Negretti breed.

M'Gowan (1925). The outbreaks have usually been associated with attempts to grade up the breeds.

(VII) IN BOTH DISEASES THE EVIDENCE POINTS TO THE DISEASE BEING RARELY, IF EVER, SPREAD BY CONTACT.

Fowl Paralysis.

Marginson and McGaughey (1931). Although spread by contact occurs the process seems to be slow and uncertain. They have observed instances in which pullets became noticeably diseased within a few weeks after they had been bought and the disease remained localised to one lot of birds.

Roberts (1935). The disease is not transmitted by contact.

Warrack and Dalling (1933). They have much evidence that the disease may be confined entirely to one group of birds while others of the same age, hatched in the same incubators at the same time and reared in the same houses and on the same land and fed on the same food and tended by the same people have always remained healthy.

Scrapie.

Gaiger (1924). No evidence of transmission by contact.

M'Fadyean (1918). The disease does not spread in spite of the closest contact between diseased and healthy sheep confined in a house.

M'Gowan (1914). No evidence of the disease being contagious as diseased and healthy can intermingle without spread.

Stockman (1913). It is improbable that mere contact in a loose-box produces the disease.

(VIII) IN BOTH DISEASES EVIDENCE HAS BEEN SUBMITTED THAT THE CAUSE OF, AND/OR A PREDISPOSITION TO, THE DISEASE IS TRANSMITTED FROM THE PARENT TO THE OFFSPRING.

Fowl Paralysis.

Asmundson and Biely (1932). The evidence presented, while not conclusive, points to the inheritance of resistance to paralysis. This is indicated by (i) difference in the proportion of paralysed

pullets in different breeds, (ii) absence of paralysis among the progeny of certain males and in certain families.

Biely et al. (1932). The data presented furnish evidence in favour of the view that resistance and susceptibility to fowl paralysis and lymphomatous tumours are inherited. The early incidence of fowl paralysis points to transmission through the egg.

Doyle (1927). The important factor in disseminating the disease is the sale of breeding stock, baby chicks or eggs for hatching from a flock in which one or more birds have shown the disease.

Kerr (1932). There is a suggestion that immunity or susceptibility to the disease are hereditary in nature. In certain outbreaks circumstances appeared to point to the eggs as the source of the infection in a hitherto unaffected flock.

Marginson and McGaughey (1931). In nine outbreaks there was strong evidence that infection was introduced through eggs, in six by young chickens.

Patterson (1936). There is a marked difference in the degree of resistance and susceptibility between chickens of the same strain and between different strains of chickens as a whole. These factors are inherited.

Roberts (1932). The appearance of fowl paralysis in a flock appears related to some factor which exists in the parent stock. Birds from one source remained healthy and those from another source became diseased though the conditions were similar.

Seagar (1933). Epidemiological investigation has shown repeatedly that, in farms previously free, the chicks hatched from bought eggs have developed fowl paralysis and on further enquiry fowl paralysis was found to exist in the parent stock from which the eggs came.

Warrack and Dalling (1932). Of 33 outbreaks studied the source of the infected birds was in six bought-in eggs, in six bought-in day-old chickens and in eleven bought-in pullets (two to six months old), while in the remaining ten the birds were hatched from the poultry farmer's own hens bred to imported stock. They have several records of the disease being confined to the progeny of one group of birds on a farm, that group having had fowl paralysis in it during their pullet year and they have record of a single hen in a flock producing diseased stock. While they have insufficient evidence that the male bird may play a part in transmission there are good grounds for believing that this may occur in some cases (in ten of 33 outbreaks the only imported stock were cockerels).

Scrapie.

Friedberger and Fröhner (1910). The disease is usually introduced into a flock by a breeding ram which is visibly affected or carries the germs of the disease.

Gaiger (1924). After the introduction of a tainted tup into a clean flock scrapie appears in his progeny at the age of two years.

M'Fadyean (1918). The disease appears in the majority of cases to be either congenital or contracted during the first few months after birth.

M'Gowan (1922). Scrapie is notoriously an hereditary disease.

Stockman (1913). The disease is possibly transmitted to the ewe or ram by the sexual act or to the lamb *in utero* owing to infection on the part of the sire or dam.

(IX) BOTH DISEASES ARE STATED TO HAVE A LONG INCUBATION PERIOD.

Fowl Paralysis.

Doyle (1926). Certain epidemiological features of the disease under farm conditions often suggest a long incubation period. For instance, birds obtained as day-olds from affected flocks often develop disease in four to eight months, while others kept under the same conditions but originating from unaffected flocks do not develop the disease.

Seagar (1933). The incubation period is usually over two months.

Warrack and Dalling (1932). The incubation period of naturally-occurring and artificially-produced disease is usually between two and six months.

Scrapie

Gaiger (1924). The disease has as its incubation period a space of over two years.

Stockman (1913). The disease very seldom appears in a visible form even in animals on infected farms or in those which have been removed from infected farms to clean ones for a period of about 18 months after exposure to what he considers to be infection.

(X) IN BOTH DISEASES THERE IS EVIDENCE THAT STOCK FROM "INFECTED" PARENTS MAY DEVELOP THE DISEASE ON ONE FARM AND NOT ON ANOTHER ("THE PROBLEM OF THE DIVIDED LOT").

Fowl Paralysis.

Biely et al. (1932). Eight to nine per cent. of chicks sold from an infected stock to another farm, where paralysis was prevalent, developed the disease. No losses have been reported by others who purchased chicks from this farm.

Warrack and Dalling (1932). In appendix is recorded a case where original stock remained healthy and chickens from that stock reared on other farms developed the disease. The original stock were under ideal conditions: the others were not.

Scrapie.

Gaiger (1924). Tainted ewe lambs taken away from the scrapie area do not develop the disease to any considerable extent. In Germany it is believed by some that the progeny of certain rams become ill in one district and not in another.

M'Gowan (1922). One hundred and sixty half-bred ewe hoggs were brought from a hill farm reputed to be free from the disease. They were taken to a farm in the neighbourhood, thoroughly jumbled up and then divided into two lots without selection. Sixty were kept on the last farm, which is a heavy clay, while 100 were sent to another farm, the soil of which is of a light nature. Of the 60 lot not one developed scrapie, while a very large percentage of the 100 lot died from scrapie.

(XI) THE METHODS OF PREVENTION ADVOCATED ARE SIMILAR IN BOTH DISEASES.

Fowl Paralysis.

Jungherr (1935). Control measures should probably take into account the selection of progeny from poultry stock of known freedom from disease and of proved viability and the control of diseases during the growing period by sanitation.

Patterson (1936). Build up resistant strains. See that new stock are of a resistant strain.

Seagar (1933). Create a foundation stock free from taint of infection and of a resistant strain.

Warrack and Dalling (1932). Eliminate diseased (latent and patent) breeding stock.

Scrapie.

Friedberger and Fröhner (1910). Adopt natural methods of keeping and feeding especially of the young. Avoid too great refinement of race by inbreeding. Use rams only after their second year.

Gaiger (1924). Keep the ewe lambs from the older ages. Use only tups over two years old.

M'Gowan (1925). Keep stock ewe lambs from the older ages only. In selecting animals for stock, robustness of constitution and family history as regards health should be considered.

SUMMARY.

A review of literature on paralysis in fowls and scrapie in sheep shows that the two diseases have many features in common.

ACKNOWLEDGMENT.

The author is indebted to the Animal Diseases Research Association for the use of its library.

REFERENCES.

- Asmundson, V. S., and Biely, J. (1932). *Can. J. Research*, vi, 171.
- Biely, J., and Palmer, V. E. (1932). *Vet. Rec.*, xii, 1302.
- Biely, J., Palmer, V. E., and Asmundson, V. S. (1932). *Can. J. Research*, vi, 374.
- Blount, W. P. (1932). *Vet. J.*, lxxxviii, 289.
- Doyle, L. P. (1926). *J. Amer. Vet. Med. Assoc.*, lxviii, 622.
- . (1927). *Ibid.*, lxxii, 585.
- Friedberger and Fröhner. (1910). *Veterinary Pathology* (Translated by Captain Hayes). Hurst & Blackett, London.
- Gaiger, S. H. (1924). *J. Comp. Path. & Ther.*, xxxvii, 259.
- Jungherr, E. (1935). *J. Amer. Vet. Med. Assoc.*, lxxxvi, 424.
- Kerr, W. R. (1932). *Vet. Rec.*, xii, 1134.
- M'Fadyean, J. (1918). *J. Comp. Path. & Ther.*, xxxi, 102.
- M'Gowan, J. P. (1914). *Investigations into Scrapie*. Blackwood & Sons, Edinburgh.
- . (1922). *Scot. J. Agric.*, v, 365.
- . (1925). *Ibid.*, viii, 190.
- Marginson, G. C., and McGaughey, C. A. (1931). *Vet. Rec.*, xi, 573.
- Pappenheimer, A. M., Dunn, L. C., and Cone, V. (1929). *J. Exp. Med.*, xlix, 63.
- Patterson, F. D. (1936). *J. Amer. Vet. Med. Assoc.*, lxxxviii, 32.
- Patterson, F. D., Wilcke, H. L., Murray, E., and Henderson, E. W. (1932). *Ibid.*, lxxxi, 747.
- Roberts, R. S. (1935). *Vet. J.*, xci, 127.
- Seagar, E. A. (1933). *Ibid.*, lxxxix, 454.
- Stockman, S. (1913). *J. Comp. Path. & Ther.*, xxvi, 317.
- Thomas, J. C., and Hamilton, H. P. (1935). *Vet. J.*, xci, 526.
- Warrack, G. H., and Dalling, T. (1932). *Ibid.*, lxxxviii, 28.

RESULTS OF PASSAGE OF HUMAN AND MONKEY STRAINS OF *BRUCELLA MELITENSIS* THROUGH PREGNANT HEIFERS.

By D. E. WILSON (*Medical Officer*)

and

S. A. EVANS (*Veterinary Research Officer*),
Tanganyika Territory.

WILSON (1935) has shown that *Brucella* infections are common in a cattle-bearing district of Tanganyika Territory. On March 8th, 1935, he isolated a strain of *Brucella* by blood culture from a typical case of undulant fever in man. This strain, No. 256, was kindly typed by Professor G. S. Wilson in London and at the Veterinary Research Laboratory, Kabete, Kenya, and reacted as a typical *Br. melitensis* when submitted to the following methods of typing:

H₂S production;
Reaction to monospecific sera;
Dye sensitivity.

During further experiments a local variety of monkey (*Cerco-pithecus* spp.) was infected with strain 256. The culture proved extremely virulent for the monkey, from which a pure culture was again recovered by seeding blood on to liver agar. The recovered organism was again typed by Professor G. S. Wilson and at Kabete as a typical *Br. melitensis* and labelled 256 M.1. On the assumption that local human infections were likely to be bovine in origin, it was decided to pass strain 256 M.1 through bovines in order to determine the effects. This was undertaken before the results of typing had been received. For this purpose, a young heifer advanced in first pregnancy was selected from a herd, all the adult members of which had been found to be free from *Br. abortus* infection as determined by agglutination. The herd was tested again eleven months later and again no positive sera were found and further, no clinical evidence of contagious abortion has ever been observed in this herd. On April 9th, 1935, the selected heifer was isolated in fly-proof quarantine quarters, subsequent to which its serum failed to agglutinate either *Br. melitensis* or *Br. abortus*. On April 12th the animal was infected *via* the conjunctival route with a few drops of a concentrated 72 hour culture of strain 256 M.1 and the following observations were made:—

The temperature curve showed a gradual and definite rise for a period of four weeks, when it reached a peak of 104·6° F. It remained about this level until the end of the fifth week, when the heifer calved to time but had a protracted and difficult labour during which the calf died. The temperature then gradually dropped and was normal a week later. There were no further rises of temperature during a further observation period of two months.

SEROLOGICAL REACTIONS.

Date.		<i>Br. melitensis.</i>		<i>Br. abortus.</i>
Apr. 16th		Neg.		Neg.
" 24th	+++	1 : 400	+++	1 : 200
May 1st	++++	1 : 400	++++	1 : 200
" 8th	++++	1 : 400		
" 15th	+++	1 : 100	(day prior to calving)	
" 22nd	++++	1 : 400		
" 22nd	±	1 : 800		
" 29th	++	1 : 1600		
June 5th	++++	1 : 1600		
" 12th	++++	1 : 1600	++++	1 : 3200
" 19th	++++	1 : 1600	++++	1 : 3200
" 26th	++++	1 : 6400	++++	1 : 6400
July 3rd	+++	1 : 6400		1 : 1600
" 10th	++++	1 : 3200		
" 17th	++++	1 : 3200		
" 24th	+++	1 : 3200	++	1 : 3200
" 31st	+++	1 : 1600		
Aug. 7th	++++	1 : 1600	++++	1 : 3200

Note : ++++ = Complete agglutination, water clear.

+++ and ++ = Varying degrees of agglutination.

± = Doubtful.

These clinical observations, together with the detailed agglutination results, are recorded for the purpose of stressing the fact that this definite syndrome was due to our own interference and not the result of previous or intercurrent infection.

From the stomach contents of the recently-born calf, the organism was recovered in pure culture. This strain (256 C.) was again typed by Professor G. S. Wilson and at Kabete and was on this occasion returned as *Br. abortus*.

The following presents the main features of this preliminary investigation in summary :—

HUMAN.

Br. melitensis 256 from blood.

MONKEY.

Br. melitensis 256 M.1 from blood.

BOVINE.

Br. abortus 256 C. from stomach contents of calf.

As this result was rather surprising and did not agree with the findings of workers such as Evans (1923), Gilman, Milk and Birch (1934) and Taylor, Vidal and Roman (1934) it was decided to repeat the experiments using the same monkey strain and a typical local human *melitensis* strain, typed by the three methods mentioned earlier in the paper.

Four pregnant heifers were selected from the herd providing the original heifer, isolated in fly-proof quarters and serologically tested for the presence of agglutinins to *Br. abortus* prior to the experiments with negative results.

Cow No. 90 received on November 5th, 1935, *via* the conjunctival route a few drops of a concentrated emulsion of the monkey strain 256 M.1. On December 12th, 1935, she calved an apparently healthy calf. A culture of *Brucella* was isolated from the calf's spleen. On typing—this culture proved to be *melitensis*.

Result : No change in type.

Cow No. 91 received on November 5th, 1935, *via* the conjunctival route a few drops of a concentrated emulsion of the monkey strain 256 M.1. On January 4th, 1936, she aborted a dead foetus aged six months. A culture of *Brucella* was isolated from the calf's spleen. On typing—this culture proved to be *melitensis*.

Result : No change in type.

Cow No. 84 received on November 7th, 1935, *via* the conjunctival route a few drops of a concentrated emulsion of the human *melitensis* strain (256). On December 28th she produced a live but feeble calf, probably of about eight months development. A culture of *Brucella* was isolated from the calf's spleen. On typing—this culture proved to be *melitensis*.

Result : No change of type.

Cow No. 85 was infected similarly to cow 84 on November 5th, 1935. On November 24th she calved a live calf which appeared to be about full term. No culture of *Brucella* was isolated from the calf.

SUMMARY AND DISCUSSION.

(1) A case of apparent mutation of *Br. melitensis* (human-monkey passage) to *Br. abortus* by passage through a pregnant heifer is recorded.

(2) Subsequent attempts to repeat this mutation failed, using the same strain and a known human *melitensis* strain.

(3) It has been suggested that the heifer used in the original experiment carried a latent *abortus* infection and that agglutination tests failed to reveal its presence. That this is possible is indicated by the work of Birch and Gilman (1931) and Kruger (1932).

(4) That the recovered organism was *Br. abortus* does not appear to be in doubt and it is equally certain that the infection instilled into the conjunctival sac was *Br. melitensis*. If the explanation of a latent infection of *Br. abortus* is accepted then the fate of the introduced *melitensis* remains unsolved. The possibility of a mixed infection of *Br. abortus* and *Br. melitensis* in the foetal tissues appears feasible if the latent *abortus* infection hypothesis be accepted, but no indication of such a phenomenon appeared in the typing.

(5) Further work on these lines, using as many animals and as many strains as possible, appears desirable. Tanganyika Territory possesses many advantages for such work, chiefly on account of the low price of the experimental animals necessary.

(6) While no final conclusions regarding the probability of mutation from type to type can be drawn from this single result, record of the experiment appears to be desirable.

We wish to record our thanks to Professor G. S. Wilson and Mr. R. Daubney, Kabete, for the work they did for us.

REFERENCES.

- Birch, R. B., and Gilman, H. L. (1931). Report N. York State Coll., 18, 56-88.
Evans, Alice C. (1923). Public Health Reports, April 20, 825-826.
Gilman, Milk and Birch. (1934). *Jl. Infect. Dis.*, **54**, 171.
Kruger, H. (1932). *Deuts. Tierärztl. Wschr.*, **40**, 481-484.
Taylor, R. M., Vidal, L. F., and Roman, G. C. R. (1934). *Soc. Biol., Paris*, **116**, 132-134.
Wilson, D. E. (1935). *East Af. Med. J.*, July, 100.

We have also to thank the respective Directors of Medical and Veterinary Services, Tanganyika Territory, for permission to publish this paper.

EXPERIMENTS ON IMMUNISATION AGAINST *PASTEURELLA SEPTICA* INFECTION.*

By F. W. PRIESTLEY,

*Department of Bacteriology and Preventive Medicine,
University of Manchester.*

THE so-called hæmorrhagic septicæmias of domestic animals caused by *Past. septica* constitute a major source of trouble in many parts of the world. In consequence many attempts have been made to produce an efficient immunising agent. Thus Pasteur (1880*a, b*), Lignières (1902), Hadley (1912, 1913, 1914*a, b*), Manninger (1919, 1920) and Harvey and Iyengar (1921-22, 1922-23, 1923) all used avirulent cultures with a variable measure of success. In a few cases cultures, whose initial virulence had been high, have been so treated that they became avirulent; for example, Szász (1919) used cultures incubated for four to five days, while Sakamoto (1922) and Meissner and Schern (1910) caused attenuation by incubating at relatively high temperatures. In general it has been shown that living cultures, while they afford a measure of protection under experimental conditions, are too unsafe for routine use in the field. Dead cultures have generally proved to be unreliable; thus Telitschenko (1911) failed to protect hens with three doses of a vaccine heated to 60° C., and Hadley and Caldwell (1919) used suspensions of virulent and avirulent strains heated at 63° C. for 30 minutes with only poor results. It is clear, however, even from the literature, that the methods of killing have not always been satisfactory; thus, for instance, Iyengar (1925-26) showed that vaccines killed at low temperatures were superior to those killed at high temperatures. (A general review of the literature on vaccination against *Past. septica* is given by Schütze [1929]. It would serve no useful purpose to requote all the literature here, and the reader is referred to Schütze's article for more details and references.)

The author (Priestley, 1936), confirming an observation of Manninger's (1919), has recently shown that the virulent variant of *Past. septica* possesses a capsule demonstrable by a special technique, and that the avirulent variant possesses no such structure. Briefly the technique is as follows: A loopful of a saline or distilled water suspension is placed on a slide, a loopful of Indian ink is added, a coverslip dropped on, and the preparation is examined with a 1/12 in. objective, using suitably graded light; the capsule appears as a distinctive halo around the organism, this area being uninvaded by the ink particles; reasons

* Owing to another appointment it has been found impossible to complete this work; it was, however, regarded as being sufficiently far advanced to warrant publication.

for believing this to be a true capsule will be found in the original article. It was further shown that this capsule is very labile, being destroyed at 56° C. in 45 to 60 minutes. Moreover, if virulent organisms are incubated for more than 24 hours there tends to be loss of capsule substance, and if incubation is carried out at temperatures much above 37° C. the capsule is never formed. It was considered likely, *a priori*, that the capsule is of importance in immunity; experiments were therefore designed to test this hypothesis.

TECHNIQUE.

The first essential in the preparation of the vaccine was to obtain variants which were reasonably constant. This was often a difficult task; it was usually necessary to plate selected colonies daily for some time; after some weeks the selected variants were fairly constant, but at first virulent and avirulent colony types appeared with confusing irregularity. On the other hand, there have been received at this laboratory occasional cultures which constantly gave rise to only one type of colony, this type not necessarily being the avirulent one. These remarks apply principally to cultures which have been isolated for some time; the author has had only a limited amount of experience with primary cultures.

Suitable cultures having been obtained, vaccine containing capsular antigen was prepared as follows: The culture was grown on serum-agar at 37° C. for 24 hours; the resultant growth was washed off with sterile saline and the suspension examined for capsules; if capsules were seen the suspension was diluted so as to have an opacity equivalent to tube No. 1 of Brown's Opacity Standards and was then placed, in narrow test tubes, into a water-bath at 56° C. for 30 minutes. The suspension was then allowed to cool at room temperature and was again examined for capsules. If capsules were demonstrable 0.5 c.c. amounts of the vaccine were inoculated intraperitoneally into a few mice to make sure that all organisms were dead. This is a precautionary measure of some importance, since the temperature of heating must be minimal consistent with a lethal effect. In this connection it is interesting to note the results of an experiment devised to test this point.

The most virulent fluorescent variant (see Hughes, 1930) available was sown on serum-agar and incubated at 37° C. for 24 hours. The growth was washed off with sterile saline and standardised to tube 2 of Brown's Opacity Standards, *i.e.* to twice the density used for immunisation purposes. This suspension was placed in the water-bath at 56° C. and at various intervals fractions were removed; these were examined for capsules by the Indian ink technique, and tested for viable organisms by the intraperitoneal inoculation of mice with 0.5 c.c. amounts. The results, shown in Table I, demonstrate that such suspensions become innocuous after 15 minutes at 56° C., whereas capsules are still evident after 30 minutes. Thus suspensions

heated at this temperature for 30 minutes may be regarded as quite safe. However, differences in thickness of glass, etc., must be taken into account and it is therefore always advisable to test the vaccine for viability before use.

TABLE I.
SHOWING THE EFFECT OF A TEMPERATURE OF 56° C. ON THE VIABILITY
AND CAPSULATION OF *Past. septica*.

Time of Heating at 56° C. (Minutes.)	Numbers Mice Inoculated.	Numbers Dying.	Examination for Capsules.
0	3	3	+
5	3	3	+
10	3	1	+
15	3	0	+
30	3	0	+
45	—	—	—

+ = Capsules demonstrated.

The other vaccines used for comparative purposes in this work were prepared in the same way as above except that in certain instances their treatment with heat was different.

Throughout, mice have been used as experimental animals, the vaccine being administered one or more times in a dose of 0.5 c.c.

The test doses given in the various experiments are referred to as so many average lethal doses (a.l.d.'s), although it is fully realised that the doses have not been determined with anything like statistical accuracy (*see* Trevan, 1927). In this connection, however, it has been noted repeatedly (by the author and others) that if the a.l.d. of a culture of *Past. septica* is estimated at a number of different times the result is always approximately the same. It follows that the labelling of a given amount of a standardised suspension as containing so many a.l.d.'s may be more trustworthy than appears at first sight. This is only true, of course, if pure variants are being used; in ordinary unselected cultures one variant type may be predominant at one time and another type at another time.

RESULTS.

Experiment 1.—Using virulent and avirulent variants of a strain of *Past. septica* isolated from a fowl, three standardised suspensions were prepared and were treated as follows:—

- (a) Virulent capsulated *Past. septica* heated to 56° C. for 30 minutes.
- (b) Avirulent non-capsulated *Past. septica* heated to 56° C. for 30 minutes.
- (c) Virulent capsulated *Past. septica* heated to 100° C. for 60 minutes.

Each vaccine was inoculated intraperitoneally in a dose of 0.5 c.c. into 40 mice. A control batch of 40 mice was collected and

kept under similar conditions. Eleven days later all surviving mice were inoculated intraperitoneally with 100 a.l.d.'s of the virulent variant.

All mice died. It was clear, therefore, that the animals had not been immunised sufficiently to withstand 100 a.l.d.'s. Those mice vaccinated with the virulent variant heated to 56° C. had a slightly higher mean survival time than the remainder, but the difference between any two groups was not greater than could be ascribed to chance.

Experiment 2.—For this experiment three vaccines were prepared as above; in addition one of *Bact. coli* heated to 100° C. for 60 minutes was included. These were each inoculated intraperitoneally into ten mice in the usual way, the immunity test being carried out 13 days later by the intraperitoneal inoculation of approximately 2 a.l.d.'s of the virulent variant. Although the numbers were small the results, shown in Table II, were distinctly encouraging. It may be seen that the survival rate in the group vaccinated with capsulated organisms is greater than that in any other group.

TABLE II
SHOWING THE EFFECT OF PROPHYLACTIC VACCINATION AGAINST *Past. septica*.

<i>Vaccine.</i>		<i>Numbers Tested.</i>	<i>Numbers Surviving.</i>
Virulent variant heated to 56° C.	...	10	7
Avirulent " "	...	10	2
Virulent " " 100° C.	...	10	3
<i>Bact. coli.</i> " "	...	10	3
Controls (unvaccinated) ...	...	10	1

Test dose = 2 a.l.d.'s.

Experiment 3 was designed to see if the resistance of mice could be raised appreciably by repeated inoculation of vaccine. Groups of mice were accordingly vaccinated intraperitoneally three times at intervals of six days with the four types of vaccine used in the last experiment. Fourteen days after the last dose of vaccine these mice, together with a control unvaccinated group, were tested for immunity against 100 a.l.d.'s of the virulent variant. The mice were kept under continuous observation for 48 hours, the time to death being noted in all cases; for purposes of estimating mean survival times the six mice which survived were regarded as having died in 48 hours. The results are recorded in Table III.

It is to be noted that the differences of 46·2 per cent. in the survival rate between the group vaccinated with capsulated organisms and the other groups are statistically significant; for example, the difference between groups 1 and 5 is 3·4 times its standard error, while between group 1 and that group containing the smallest number of animals, namely group 3, the difference is 2·6 times its standard error.

TABLE III.
SHOWING THE EFFECT OF REPEATED VACCINATION AGAINST *Past. septica*.

Vaccine.	Nos. Tested.	Nos. Surviving.	Per cent. Surviving.	Mean Survival time. (Hours.)
1. Virulent variant heated to 56° C.	13	6	46.2	37.0 ± 3.4
2. Avirulent " " " "	20	0	0	27.3 ± 1.9
3. Virulent " " " 100° C.	11	0	0	24.2 ± 1.9
4. <i>Bact coli</i> " " " "	17	0	0	22.7 ± 1.5
5. Controls (unvaccinated) ...	20	0	0	18.7 ± 1.0

Test dose = 100 a.l.d.'s.

As regards survival times, it is not surprising to find that group 1 shows a significantly higher mean value than the control group 5, the difference, 18.3 hours, being 5.1 times its standard error. But it is perhaps surprising to find that the mean survival times of groups 2, 3 and possibly even 4 are significantly greater than that of the controls. In the latter case, *i.e.*, the group vaccinated with *Bact. coli*, the difference, which is 2.2 times its standard error, can only be ascribed to a non-specific cellular reaction in the peritoneal cavity. Finally, it is noteworthy that the mean survival time of group 1 is significantly greater than those of groups 2, 3 and 4, as well as that of the controls.

Experiment 4 was a repeat of *Experiment 3*, except that owing to lack of time the three doses of vaccine were given at intervals of two days and no groups vaccinated with *Bact. coli* or the virulent variant heated to 100° C. were included. Fifteen days later these mice were tested in the usual way against 100 a.l.d.'s. The results, which confirm those of *Experiment 3*, are shown in *Table IV*. Again it is to be noted that the differences in the survival rate between that group vaccinated with capsulated organisms and the other groups are statistically significant.

TABLE IV.
SHOWING THE EFFECT OF REPEATED VACCINATION AGAINST *Past. septica*.

Vaccine.	Nos. Tested.	Nos. Surviving
Virulent variant heated to 56° C. ...	20	7
Avirulent " " " " ...	20	0
Controls (unvaccinated) ...	20	0

Test dose = 100 a.l.d.'s.

Experiment 5.—For this experiment a strain with a less virulent fluorescent variant was used; this was in fact strain 2 referred to in the previous publication. Three groups of mice were formed; one group was vaccinated subcutaneously with the virulent variant heated to 56° C. for 30 minutes, one group with the avirulent variant treated in the same way, and one group was kept as controls. Two doses of vaccine were given with an interval

of seven days between each. Fourteen days after the last dose the mice were divided into two groups, one group being tested by the intraperitoneal inoculation of approximately 1 a.l.d. and the other by the inoculation of 10 a.l.d.'s.

TABLE V.
SHOWING THE EFFECT OF VACCINATION AGAINST A VARIANT OF *Past. septica*
OF RELATIVELY LOW VIRULENCE.

Vaccine.	Test dose = 1 a.l.d.			Test dose = 10 a.l.d's.		
	Nos. Tested.	Nos. Surviving.	Per cent. Surviving.	Nos. Tested.	Nos. Surviving.	Per cent. Surviving.
Virulent variant heated to 56° C.	10	9	90	13	11	84·6
Avirulent variant heated to 56° C.	5	5	100	10	5	50·0
Controls (unvaccinated)	... 10	1	10	10	1	10·0

The results recorded in Table V suggest that, when a strain of relatively low virulence is used for test purposes, vaccines prepared from avirulent organisms are quite efficient if the test dose is not large; the superiority of vaccines prepared from capsulated organisms only becomes apparent when larger test doses are employed.

As a conclusion it is interesting to note the fate of three rabbits which received an inoculum (1×10^6 mouse a.l.d.'s) of living virulent variant during the course of immunisation. One of these rabbits had been immunised with virulent variant heated to 56° C.; this animal, subsequently given the living dose, survived in perfect health. The two other rabbits had been immunised against the avirulent variant and their sera contained a relatively high concentration of somatic antibody; on inoculation with the living virulent variant one rabbit died within 48 hours, while the other developed snuffles and eventually succumbed after some weeks of illness. By themselves these observations mean little, but they may be regarded as confirmatory evidence.

DISCUSSION.

The results reported in this paper support the hypothesis that the capsular antigen is of importance in immunisation against *Past. septica*. Vaccines prepared from organisms killed in such a way that capsule is not destroyed have been shown to be superior to those prepared from organisms containing no capsule. The somatic antigen probably plays only a minor part; at any rate it is clear that this antigen alone is insufficient to engender in mice an immunity capable of rendering them resistant to highly virulent cultures. When the test organisms are less virulent, or what is

perhaps synonymous, when they are less well protected by capsule immunisation with somatic antigen alone may be partially successful, but even under these conditions the additional use of capsular antigen substantially increases the immunity.

To obtain a fair comparison with the results of previous workers it would have been necessary to prepare vaccines from organisms heated to about 60° C. Heating at 100° C. was perhaps a little drastic, but this temperature was adopted for abolishing capsule in order to avoid confusion of the issue since it has been shown by Schütze (1932) that the haptene of the capsular antigen of *Past. pestis* may still be in a reactive state after heating at 100° C. for 15 minutes, and Zozaya and Clark (1933) and others have shown that under certain conditions small amounts of polysaccharide may act antigenically and stimulate the production of antibodies. Even so, the many failures that have been reported in the literature may now perhaps be explainable. It is clear that in many instances, for example, where avirulent cultures have been used, no capsular antigen has been present. In other instances, for example, where initially virulent cultures have been attenuated, the processes of attenuation must often have resulted in loss of capsule substance; this would be the case with cultures which have been incubated either too long or at too high a temperature. Similarly, it is now evident that dead vaccines have generally been prepared by methods destructive to capsular antigen; it has been the usual practice to kill by heating at temperatures of 60° C. or more. It is noteworthy that as far back as 1925 Iyengar had shown the desirability of low temperature heating; however, as the rationale of this procedure was not then evident this observation did not attract the attention it deserved.

The important points to note then, in connection with the preparation of an efficient vaccine against *Past. septica* infection are: first, that a highly virulent and markedly capsulated strain of organism should be used; second, that the time and temperature of incubation should be optimal for capsule formation; and third, that suspensions should be treated in such a way that while the organisms are killed the capsular antigen is unharmed. In the work reported here it will be noted that the organisms have been suspended in saline, no preservative of any kind having been added; clearly for field purposes it will be necessary to add preservative to the vaccine, but this point may need some study since it has been shown that certain antigens of importance in immunisation may be radically altered by unsuitable preservatives (see Felix and Bhatnagar, 1935).

SUMMARY.

A method of preparing a vaccine from virulent capsulated *Past. septica* is described. It is shown that this vaccine is able to protect mice against a dose of organisms sufficient to kill all

controls and all mice inoculated with the type of vaccine containing only somatic antigen.

REFERENCES.

- Felix, A., and Bhatnagar. (1935). *Brit. J. Exp. Path.*, **16**, 422.
 Hadley, P. (1912). *Z. Bakt., Abt. 1, Orig.*, **61**, 323.
 —. (1913). *Ibid.*, **69**, 271.
 —. (1914a). *Bull. R.I. Agric. Expt. Sta.*, **157**, 285.
 —. (1914b). *Ibid.*, **159**, 384.
 Hadley, P., and Caldwell, D. W. (1919). *Ibid.*, **179**, 4.
 Harvey, W. F., and Iyengar, K. R. K. (1921-22). *Ind. J. Med. Res.*, **9**, 730, 736.
 — and —. (1922-23). *Ibid.*, **10**, 192, 424, 990, 1147.
 — and —. (1923). *Ibid.*, **11**, 110, 113, 433.
 Hughes, T. P. (1930). *J. Exp. Med.*, **51**, 225.
 Iyengar, K. R. K. (1925-26). *Ind. J. Med. Res.*, **13**, 547, 917.
 Lignières, J. (1902). *C.R. Acad. Sci., Paris*, **134**, 1169.
 Manninger, R. (1919). *Z. Bakt., Abt. 1, Orig.*, **83**, 520.
 —. (1920). *Deuts. tier. Wschr.*, **29**, 543.
 Meissner and Schern. (1910). *Arch. wiss. prakt. Tierhkl.*, **36**, 44, 208.
 Pasteur, L. (1880a). *C.R. Acad. Sci., Paris*, **91**, 239.
 —. (1880b). *Rec. Med. vet.*, **180**, 125, 422.
 Priestley, F. W. (1936). *Brit. J. Exp. Path.*, **17**, 374.
 Sakamoto, K. (1922). *J. Jap. Soc. Vet. Sci.*, **1**, 151.
 Schütz, H. (1929). M.R.C. A system of Bacteriology. (London.) Vol. 4.
 —. (1932). *Brit. J. Exp. Path.*, **13**, 284.
 Szász, A. (1919). *Z. Infekt. Kr.*, **20**, 26.
 Telitschenko, E. (1911). See *Z. Bakt., Abt. 1, Ref.*, **51**, 446.
 Trevan, J. W. (1927). *Proc. Roy. Soc. B.*, **101**, 483.
 Zozaya, J., and Clark, J. (1933). *J. Exp. Med.*, **57**, 21.

A NOTE ON THE ASSOCIATION OF THE VIRULENCE OF *PASTEURILLA SEPTICA* WITH CAPSULATION.

By F. W. PRIESTLEY,
South-Eastern Agricultural College, Wye, Kent.

MANNINGER (1919), using a special technique involving the use of Indian ink, was apparently the first to note that virulent variants of *Past. septica* were capsulated while avirulent variants were not. This observation has recently been confirmed and extended by Priestley (1936a, b). A point of considerable importance in demonstrating the capsule was found to be the time of incubation of cultures; after incubation at 37° C. for 24 hours it is fully developed; after 48 hours it is still present but is distinctly smaller; after 72 hours it is generally absent, only a few strains retaining it until the fourth day. It was considered likely that as the capsule disappeared, as a result of prolonged incubation, so the organisms

TABLE I
DEMONSTRATING THE RELATION BETWEEN CAPSULATION AND VIRULENCE
OF *Past. septica*.

Suspensions Prepared from	Mice Inoculated Intraperitoneally with 0.5 c.c. Suspension Diluted 1 in :								
	10	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷	10 ⁸	10 ⁹ to 10 ¹²
Virulent organisms grown 1 day	++	++	++	++	++	++	++	+-	—
Virulent organisms grown 2 days	++	++	++	+-	+-	++	++	—	—
Virulent organisms grown 3 days	++	++	++	++	++	—	—	—	—
Virulent organisms grown 4 days	++	++	+-	—	—	—	—	.	.
Avirulent organisms grown 1 day	—	—	—	—	—	—	—	.	.
	Blood Broth Inoculated with 0.5 c.c. of Suspension Diluted 1 in :								
	10	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷	10 ⁸	10 ⁹ to 10 ¹²
Virulent organisms grown 1 day	+	+	+	+	+	+	+	+	—
Virulent organisms grown 2 days	+	+	+	+	+	+	+	+	—
Virulent organisms grown 3 days	+	+	+	+	+	+	+	—	—
Virulent organisms grown 4 days	+	+	+	+	+	+	—	.	.
Avirulent organisms grown 1 day	+	+	+	+	+	+	+	.	.

++ = both mice died. +- = only one mouse died. + = growth.
— = neither mouse died. . = not done. — = no growth.

would become less virulent. This hypothesis was therefore put to the test in the following way:—

Saline suspensions were prepared from virulent organisms grown on blood agar at 37° C. for one, two, three and four days, and from avirulent organisms similarly grown for one day. The strain used was highly virulent under optimal conditions and was, moreover, one of the few which retain a faint degree of capsulation after four days' incubation. Each suspension was examined for capsules, the results being in accordance with expectation. The suspensions were then standardised by opacity to tube 1 of Brown's Opacity Standards, and serial 1 in 10 dilutions were made, using a different pipette for each dilution. 0.5 c.c. of each dilution was then sown into blood broth and a similar quantity inoculated intraperitoneally into each of two mice.

The results, shown in Table I, are unequivocal. They show that virulence decreases as the capsule disappears. That this loss of virulence might possibly be due to a decrease in the number of viable organisms was anticipated and controlled; it may be seen that with a virulent culture incubated for one day the minimum number of organisms necessary for commencement of growth in blood broth is the same as the minimum number necessary to kill a mouse; with a two-day culture ten times the amount necessary for growth is necessary to kill a mouse; with a three-day culture 100 times and with a four-day culture 1,000 times this amount is necessary. This ten-fold reduction every 24 hours is perhaps fortuitous but the general indication is clear enough.

It is interesting to note that, while initially virulent cultures incubated at 37° C. for four days become relatively avirulent they do not lose their power of producing capsule and thus becoming fully virulent again. It follows—and this has been a general finding—that the use of cultures attenuated in this way for immunisation must be a procedure attended with some risk.

REFERENCES.

- Manninger, R. (1919). *Zlb. Bakt., Abt. 1, Orig.*, **83**, 520.
Priestley, F. W. (1936a). *Brit. J. Exp. Path.*, **17**, 374.
——. (1936b). *J. Comp. Path. & Therap.*, in press.

THE VALUE OF VACCINATION WITH LIVE CULTURE OF BR. ABORTUS FOR THE PREVENTION OF ABORTION.

By Major H. E. KEYLOCK, F.R.C.V.S.

Shanghai.

November 1933.

DURING my visit to England in this year I called on Mr. P. J. L. Kelland, Chief Veterinary Officer, Ministry of Agriculture and Fisheries, London, to ascertain if he thought it advisable to use live abortion vaccine in the dairy. Mr. Kelland asked me to call when Dr. W. H. Andrews, the Director of the Veterinary Laboratory, Ministry of Agriculture and Fisheries, Weybridge, would be present. I took advantage of this invitation and in order that Mr. Kelland and Dr. Andrews could form some opinion of the incidence of the disease in the dairy I submitted to them the following extract from our records.

Year Ending.	Average Number Cow in Milk per Day.	Per cent. of Normal Calvings.	Per cent. of Abortions.	Per cent. Barren.	Per cent. of Effi- ciency.	Per cent. discarded for Steri- lity.	Per cent. discarded for Tubercu- losis.	Average Yearly Milk Yield per Cow.
1924		53.41	14.66	28.80	68.06			
1925		29.56	26.11	40.89	56.16			
1926	171	37.63	21.66	37.64	59.28			4920
1927	221	36.14	10.41	50.50	36.63			3447
1928	233	42.71	17.19	35.42	59.90	3.50	8.17	5724
1929	255	42.66	18.35	36.70	61.01	5.84	8.45	6133
1930	283	37.82	16.80	40.34	58.40	4.71	5.23	6607
1931	350	30.79	21.27	43.81	52.06	4.01	6.33	6007

Normal Calvings.—Produced a calf at full term within one year.

Aborted.—Aborted during the year.

Barren.—Neither aborted nor produced a calf. Some may have aborted a very small foetus.

Efficiency.—Conceived, *i.e.*, either produced a full term calf or aborted.

Discarded for Sterility.—Unable to conceive; many have diseased generative organs.

The conclusions I drew from my discussions with Mr. Kelland and Dr. Andrews were:—

(1) That their observations in England with regard to the use of a vaccine composed of the living *Br. abortus* led them to believe that such use would reduce the cases of abortion in our dairy.

(2) As all the milk issued by the dairy was pasteurised, if the pasteurisation was efficient there could be no danger of the milk carrying living *Br. abortus* bacilli.

(3) The Ministry of Agriculture Laboratories could not supply the vaccine we should require and I should consider

the possibility of obtaining such vaccines from Dr. A. L. Sheather.

(4) That the agglutination test should be applied to ascertain what percentage of the animals were affected.

As there is no doubt but that our milk is efficiently pasteurised I decided to at least test the value of an injection of the live vaccine upon a fair number of cattle. I therefore wrote to Dr. Sheather and discussed the question with him. Dr. Sheather agreed to supply the vaccine and we decided that the first point to be settled was, would the organisms retain their vitality for the period necessary to send them to Shanghai, *viz.*, six weeks? A series of tests made by Dr. Sheather showed that sealed ampoules containing the vaccine were still virulent after nine weeks' storage at ordinary room temperature. I then decided to send to Shanghai 50 doses per month, and a cultural bacteriological test has proved that the first two shipments retained their vitality after arrival in Shanghai and were certainly virulent when injected, as the injections were made at once.

The arguments for and against the use of the living *Br. abortus* vaccine have received careful personal consideration and I have accepted the latest and most important contentions of each school of thought contained in the following articles:—

1.—Vaccination against Bovine Contagious Abortion and the Relation of this Disease to Undulant Fever of Man, by W. H. Andrews, D.Sc., M.R.C.V.S., Presented at Experimental Pathology Section, Folkestone, August, 1932.

2.—Vaccination against Contagious Bovine Abortion by Sir John McFadyean, *Journal of Comparative Pathology and Therapeutics*, Vol. xlyi, 1, March, 1933.

3.—Contagious Abortion in Denmark, by C. O. Jensen, same journal and issue as 2.

4.—Continuance of the discussion on No. 1 above, as held at the N.V.M.A. Congress, *Veterinary Record*, October 14th, 1933, p. 1015.

It appears to me that the principal contentions as to whether we should apply this vaccine to our dairy herd may be briefly summed up as follows:—

Those against the use of the living vaccine state that it may increase the number of animals discharging *Br. abortus* in the milk, thereby increasing the risk of infecting milk consumers; that no satisfactory proof has been established that the incidence of abortion in a herd has been reduced by the application of the vaccine; that if any satisfactory statistics in favour of the vaccine were in existence they would be presented; and that as far as present knowledge goes the best method is to ascertain the infected animals by the agglutination test and eradicate the disease by isolation or eviction of the reactors.

Those advocating the use of the vaccine allege that the Ministry of Agriculture would not be issuing per annum 25,000

doses of the vaccine for 20 years if the users of the vaccine did not obtain some benefit from its use; that eradication by the agglutination test and isolation may be effective under favourable environmental conditions but is generally impracticable; that in some herds abortions have been arrested after applying the vaccine, and that in some herds it is the only known method that can be applied and it may be successful.

Assuming that the above accurately sums up the contentions for and against the use of living vaccine, it is now necessary to view their application in the conditions existing in our own herd.

The first consideration is the belief common to both sides that the agglutination test and isolation of reactors may be successful. This would entail the division of our dairy herd into two distinct portions with no communications between them, and that is not possible for economic reasons. Also we must import dairy cattle from abroad, and importations have arrived infected with contagious abortion although the agglutination test was applied before shipment. Apropos this method of arresting any disease it is in my opinion inapplicable in this country, where nearly every infectious animal disease is encountered. Apparently organisms which cause infectious diseases will continue to exist in the world as long as there is a suitable soil upon which they can develop; therefore, even given a herd free from any disease, there can be no absolute security from infection. For this reason the safest solution must be to be in a position economically to arrest and eradicate a disease from a herd if and when it is attacked. The agglutination test with isolation is purely defensive, but an attack is the best defence.

The next point is the relationship of *Br. abortus* to undulant fever in man and the danger of conveying this disease to man by means of the milk. I think it is generally agreed that efficient pasteurisation (raising the temperature of milk to 145° F. and holding it at that temperature for 30 minutes) destroys the *Br. abortus* organism, and as all this dairy's milk receives such treatment this objection does not arise.

Having thus arrived at the conclusion that the use of live vaccine in this herd would not involve any danger to the milk-consuming public, the question of its possible effect upon dairy cattle had to be considered. I gathered that there would be no reaction to the injection of the vaccine even if a double dose were given (I have not yet ascertained the approximate number of organisms generally given with one injection). As it was not thought that the injection of live vaccine increases the number of abortions in a dairy herd we did not expect more cases to follow vaccination in our dairy than we had had during the past five years. On the other hand it is generally admitted that in some herds the disease is totally arrested after a vaccine injection. The claim has been made that sterility in some instances follows the

use of the vaccine. This claim is not stressed by anyone and others state that the reverse is usual.

It is not clear what is actually meant when an animal is said to be sterile. Literally it should mean that an animal is incapable of conceiving, but it is extremely doubtful if this is the case with a very large number of animals described as sterile. I am sure the English farmer would discard a cow as sterile not because she cannot conceive but because she has failed to conceive from previous services and it would not be economical to serve her again and keep her for a further nine months. As far as this dairy is concerned we continually serve a cow provided there is no demonstrable disease of the generative organs and for as long as she is yielding a remunerative quantity of milk. When the milk yield is insufficient and she is still barren she is discarded as sterile and is so classified, but this date may be even three years after the last pregnancy. This is the reason why we have such a comparatively small percentage discarded for sterility.

Holding the foregoing views it appeared advisable to use the living vaccine and ascertain the exact results of its application to our dairy herd, and with that object to keep such accurate records as would definitely prove its value in reducing the incidence of abortion.

How, it may be asked, can we best ascertain the value of vaccination?

Sir John M'Fadyean (*loc. cit.*) says: "The value of vaccination must be measured by the extent to which it reduces the number of abortions and prevents fresh infections in the herd."

"What would have been the incidence of abortion in the herd if the cows and heifers had not been vaccinated?"

These quotations are not all Sir John said in this connection, but I think our herd records before and after the use of vaccine should supply an answer to these questions.

Our herd records up to this date bearing upon the question are shown in the tables.

The percentage of abortions, barrenness, etc., can never balance because 1 or 2 per cent. of the dairy breds or purchases may die or abort, also a cow may abort twice in one year.

The dairy breds and purchases are excluded from the calculations of percentages because 98 per cent. of them will not abort, die, or be barren, but will produce a normal calf and these 98 per cent. are therefore efficient.

It is after an animal has been in the dairy for one year that troubles begin and it is the average losses from these troubles that we require.

It is to be noted that the returns up to November 1st, 1925, are probably incorrect, but they are correct from that date. Also note that from November 1st, 1926, to June 30th, 1927, is a period of eight months and not twelve months as are the other periods.

	(8 months)							
	Nov., 1922, to Oct., 1923.	Nov., 1923, to Oct., 1924.	Nov., 1924, to Oct., 1925.	Nov., 1925, to Oct., 1926.	Nov., 1926, to June, 1927.	July, 1927, to June, 1928.	July, 1928, to June, 1929.	July, 1929, to June, 1930.
Number on first day of year	...	108	191	203	194	202	218	278
" of Dairybreds added	...	66	43	25	23	13	36	26
" Purchases added	...	64	—	—	31	52	94*	78
" Deaths and Discards	...	47	31	34	46	33	70	67
" on last day of year	...	191	203	194	202	192	218	315
" of normal calvings	...	130	145	86	127	96	183	235
" Abortions	...	18	28	53	42	21	33	48
" Barren...	...	85	55	83	73	102	68	96
Percentage of Deaths and Discards	...	37.69	15.71	16.75	25.57	16.34	23.85	21.85
" old animals died	...	...	...	...	...	18.36	8.33	3.85
" Dairybreds died	...	...	...	...	...	0.00	27.78	11.86
" Purchases died	...	...	...	...	...	5.77	10.21	9.74
" normal calvings	...	0.00	53.41	29.56	37.63	36.14	42.71	37.82
" Abortions	...	16.66	14.66	26.11	21.66	10.41	17.19	18.35
" Barren	...	78.71	28.80	40.89	37.64	50.50	35.42	16.80
" efficiency	...	16.16	68.06	56.16	59.28	36.63	61.01	40.34
" rinderpest deaths	...	...	...	...	...	...	59.90	43.81
" tuberculosis	...	...	...	...	...	...	52.06	45.67
" sterility	...	...	...	...	...	...	0.64	50.66
" pleuro-pneumonia	...	...	...	...	...	...	8.17	2.27
" other causes	...	...	...	...	...	...	3.50	6.33
"	...	...	...	...	...	...	4.01	3.73
"	...	...	...	...	...	...	4.22	7.22
"	...	...	...	...	...	...	3.11	4.87
"	...	...	...	...	...	...	6.28	6.17
"	...	...	...	...	...	...	15.07	15.07

N.B.—Forty purchases arrived on the last day of the year (June 29th, 1929), therefore they are not considered when working out the average, but they must be included as far as numbers are concerned. As eight of these purchases died during the following year, their first year, they affect the percentages for year ending 1930 and as far as percentages are concerned they are included as purchases during the year 1929 to 1930.

EXPLANATORY NOTES.

Methods of obtaining percentages.

Percentage of Deaths and Discards is number on first day of year divided into number of Deaths and Discards.

Percentage of Barren is number on first day of year divided into number of Barren.

Percentage of Normal Calvings equals number on first day of year divided into number of Normal Calvings, less number of Dairybreds added and less number of purchases added.

Percentage of Abortions is number on first day of year divided into number of abortions.

Percentage of Efficiency is number on first day of year divided into number of Normal Calvings plus number of Abortions less Dairybreds and Purchases.

Percentage of other specific diseases is number on hand first day of year plus Dairybreds added and plus Purchases added divided into diseases concerned.

TABLE I. (Adult Animals.)

Number of Animals.	Pregnancy "A."			followed by			Pregnancy "B."		
	No.	Per-centage.	Full Term Calf.	Aborted.	Retained Placenta.	Bulls Used.	Number of Services.	*Proved Sterile.	Died and/or Discarded.
	No.	Per-centage.	No.	Per-centage.	No.	No.	No.	No.	No.
24	21	87.50	3	12.50	4	16.67	27	30	—
28	26	92.85	2	7.15	4	14.28	28	33	—
30	25	83.33	5	16.67	6	20.00	48	65	2
30	24	80.00	6	20.00	11	36.67	51	64	1
30	24	80.00	6	20.00	12	40.00	42	61	1
5	3	60.00	2	40.00			9	12	3
23	20	86.96	3	13.04	6	26.09	40	54	1
21	10	47.62	7	52.38	7	52.38	36	52	1
10	4	40.00	6	60.00	2	20.00	17	26	1
23	12	52.17	11	47.83	7	30.43	33	44	3
224	169	75.45	51	22.55	59	26.34	331	441	14
									20
									9
									181
									142
									33
									18.23
									43
									23.75

TABLE II. (Maiden Heifers.)

Heifers	Pregnancy "A."			followed by			Pregnancy "B."		
	No.	Per-centage.	Full Term Calf.	Aborted.	Retained Placenta.	Bulls Used.	Number of Services.	*Proved Sterile.	Died and/or Discarded.
30	23	76.92	7	23.08	4	17.39	26	37	1
27	24	88.89	3	11.11	6	22.22	28	42	2
12	8	66.67	4	33.33	1	8.33	9	14	1
14	11	78.57	3	21.43	2	14.29	19	26	2
83	66	79.52	17	20.48	13	15.48	107	133	6
									3
									61
									54
									88.52
									7
									11.48
									9
									14.75

*Either proved sterile or has not produced a calf within 18 months from vaccination.

TABLE III.

	Full Term Calf.		Aborted.		Retained Placenta.		Barren.		Sterile.		Died or Discarded.		Total.
	Not Vacc.	Vacc.	Not Vacc.	Vacc.	Not Vacc.	Vacc.	Not Vacc.	Vacc.	Not Vacc.	Vacc.	Not Vacc.	Vacc.	
	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.
1st year	90.92	92.86	9.08	7.14	12.92	17.86	—	—	0.13	—	7.67	—	7.67
2nd year	45.33	55.22	20.99	17.91	19.53	20.89	33.67	26.86	2.33	—	12.10	0.15	12.25
3rd year	50.00	69.66	18.75	13.48	22.35	29.21	31.25	16.85	7.07	—	15.34	0.22	15.56
4th year	51.05	65.55	12.04	13.33	17.80	17.78	36.91	21.11	7.07	3.33	23.30	13.33	36.63
5th year	54.91	75.00	9.37	7.14	20.53	37.51	35.71	17.86	4.02	3.57	31.25	10.71	41.96
6th year	47.33	57.14	11.45	19.05	25.19	23.81	41.22	23.81	7.63	—	31.30	4.76	36.06
7th year	51.85	50.00	11.11	25.00	16.05	—	37.04	25.00	6.17	—	33.38	—	33.33
8th year	45.28	—	13.21	—	18.87	—	41.50	—	18.87	—	32.07	—	32.07
9th year	42.85	—	8.57	—	22.85	—	48.57	—	22.85	—	37.14	—	37.14
10th year	27.27	—	—	—	9.09	—	59.09	—	45.45	—	54.54	—	54.54
11th year	44.44	—	13.64	—	33.33	—	55.55	—	11.11	—	11.11	—	11.11
12th year	37.50	—	—	—	37.50	—	62.50	—	50.00	—	100.00	—	100.00

TABLE IV.

	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.	Per-centage.
1st year	85.42	80.34	14.58	19.66	15.77	22.08	—	—	0.89	—	7.74	—	7.74
2nd year	41.42	43.59	19.40	19.23	20.89	14.10	39.18	37.18	3.36	1.28	14.18	2.56	16.74
3rd year	48.78	56.67	16.58	16.67	25.85	23.30	34.63	26.66	8.29	6.66	25.36	13.33	38.69
4th year	45.31	66.67	11.72	20.00	17.19	26.66	42.96	13.33	7.81	6.67	31.25	6.67	37.92
5th year	50.00	57.59	11.84	—	18.42	23.33	38.16	41.67	13.16	—	35.53	—	35.53
6th year	54.54	—	4.32	50.00	15.91	50.00	40.91	50.00	2.16	—	8.86	50.00	58.86
7th year	14.81	—	3.70	75.00	7.41	25.00	81.48	25.00	37.04	23.00	59.26	25.00	84.26
8th year	33.33	—	—	—	22.22	—	55.55	100.00	33.33	—	66.67	100.00	166.67
9th year	33.33	—	—	—	33.33	—	66.67	—	33.33	—	100.00	—	100.00

By percentage of efficiency is meant the percentage of animals that conceive, *i.e.*, either produce a full-term calf or an aborted calf during the period of twelve months.

It appears to me that the foregoing figures over a period of eight years (I am not certain of the accuracy of the figures previous to year ending 1926) will supply an answer as to the incidence of abortion if the cows were not vaccinated, and that similar figures and records kept during the next three years, during which period the cows will be vaccinated, will in comparison with the last eight years measure the extent of abortion reduction, if any.

December 1935.

The first injection with the viable abortion vaccine was made on November 14th, 1933.

As regards the potency of the vaccine, the latter was sent to Shanghai, and when returned to Dr. Sheather in England was still found to be viable, and in view of the local and temperature reactions given by the majority of the cattle it was not considered necessary to make any more tests as to its viability.

It has been claimed that no reaction follows an injection of living abortion bacilli but that has not been our experience. Some local and/or temperature reactions are very decided, but there is very little, if any, reduction in the milk yield. No abscesses have arisen at the injection site and generally the local reaction disappears within seven days, but in a few instances the vaccine takes a considerable time to become absorbed, a hard enlargement of the size of a walnut persisting for some months. In a few isolated cases the vaccine has caused considerable systemic disturbance, the animal losing condition, with an irregular appetite, some anæmia and general depression. These conditions commence slowly, persist for some time and are followed by complete recovery without treatment. We have only observed these conditions in vaccinated animals and can only attribute them to the effects of the vaccine. Temperature and local reaction have occurred in animals that have aborted previous to the injection of vaccine.

Table I.—This table shows that of 224 animals before vaccination 74·45 per cent. produced a full-term calf, 22·55 per cent. aborted and 26·34 per cent. retained their placenta. These 224 animals were then vaccinated, 14 proved sterile, 20 have not yet calved, but some may be in calf, 9 have died or were discarded, leaving a total of 181. Of this number after vaccination 81·77 per cent. produced a full-term calf, 18·23 per cent. aborted and 23·75 per cent. retained placenta.

It had to be determined what constituted an abortion. As the date of coition and fecundation is not necessarily the same the number of gestation days cannot be taken, and the classical symptoms or indeed any symptoms of an abortion (except the general appearance of the calf) are not always present. Dictionary definitions of abortion vary and the term used here means a calf expelled before it should have been in comparison with a normal full-term calf. The practical observant eye is not likely to make an error as to whether a calf has been carried its full term or not. It has therefore been upon the general appearance of the calf that a decision has been arrived at in those cases where leading symptoms were absent.

CONCLUSIONS.

Table I: Adult Animals.

If these figures can be taken at their face value then vaccination increased the number of full-term calves by 6.32 per cent., decreased abortion by 4.32 per cent. and decreased the retention of the placenta by 2.59 per cent.

It is thought that the difference in favour of vaccination is so small that figures kept over a further length of time might easily be reversed and that vaccination confers very little, if any, immunity.

Table II: Heifers.

There are no previous records for comparison, but we have never had so many abortions amongst heifers, and the number that have proved sterile is far above our previous experience, when, in fact, it has been the exception to find a sterile heifer. This sterility we can only attribute to the vaccine and as we have discontinued it on heifers subsequent records will prove this point.

Tables III and IV.

These tables are so far incomplete but they may be taken as complete up to and including the fifth year. As far as full-term calves, abortions and retained placenta are concerned, the figures are not impressively in favour of vaccination, but there seems to be a decided advantage as regards barrenness, sterility and deaths and discards. In this connection it is felt that there is some factor which we have not yet realised and that a definite decision on this subject should be left until the completed records are available at the end of this year.

We have discontinued the vaccination of any cattle, confining our efforts to control the disease by redoubling our efforts for the disinfection of the maternity boxes and all animals after calving.

Attempting to view the value of the live vaccine from another angle, the dairy records show the following:—

<i>Year.</i>	<i>Normal Calvings.</i>	<i>Abortions.</i>	<i>Percentage of Abortions.</i>
1925/26	139	47	25.3
1926/27	126	34	21.2
1927/28	144	42	22.6
1928/29	176	47	21.1
1929/30	217	43	16.6
1930/31	253	74	22.7
1931/32	312	112	26.5
1932/33	332	115	25.8
1933/34	342	121	26.2
1934/35	331	98	22.84

We commenced to use the live abortion vaccine in November 1933, and if it possessed any value the percentage of abortions should have been reduced for the year 1934-35, but there is little difference.

THROMBO-ANGIITIS OBLITERANS.

By E. D. TELFORD, F.R.C.S.,

Department of Clinical Research in Disorders of the Autonomic Nervous System, Manchester Royal Infirmary.

It has been known for many years that circulatory disturbances associated with painful lameness and in some cases with gangrene, occurred in the lower limbs of men in early and mid-adult life. Such cases were described from time to time under names such as juvenile gangrene, presenile gangrene, and endarteritis obliterans. It was recognised that the condition occurred at an earlier age than does arteriosclerosis and that the vessels did not show the histo-pathology of atheroma or calcification.

In 1924 Buerger¹ of New York published his work and from that time the disease has been widely recognised under the name of Thrombo-angiitis obliterans or Buerger's disease. The essential feature of the disease is the occurrence of thrombosis beginning in medium-sized arteries of the leg, *e.g.*, anterior and posterior tibial arteries, from which point the thrombosis is liable to spread in both upward and downward directions, converting the arteries into fibrous cords. In a few of the cases a similar change is seen also in the veins.

Section of the artery shows that its lumen is plugged by fibrous tissue resulting from the organisation of the clot and that the middle and outer coats are also infiltrated by dense fibrous tissue. This fibrous tissue will be found in severe cases to enwrap the adjacent vein and nerve into one dense fibrous packet.

So grave a change in the lumen of the arteries must lead to serious symptoms and of these the intermittent painful lameness (claudication) is the most striking and is the one which usually causes the patient to seek advice. The victim of claudication sets out to walk at his usual pace but in perhaps 200 yards will be brought to a standstill by very severe pain in the calf of the leg. He rests for two to three minutes and if he tries to continue his walk he is again pulled up within the same or perhaps shorter distance. The more severe cases develop a rest pain and some 50 per cent. of the patients will eventually come to amputation for gangrene. Of the cause of the condition we know nothing definite. It occurs in men of all races, it is singularly rare in women; tobacco and exposure have been suggested as causes but without convincing proof.

Its main symptom—claudication—is known to occur in horses. Hutyra and Marek² give a dramatic description of this. In the horse, as in man, it is of course due to a deficient supply of arterial blood to muscles in action and this deficiency is due to thrombotic occlusion of arteries. Thrombosis in the horse may be

the result of larval infection of the arterial coats—of arteriosclerosis and of embolism. These morbid processes have, however, no share in the disease of thrombo-angiitis obliterans in man. The real exciting cause would appear to be some toxic factor as yet unfortunately unknown. There are reasons to believe that the same disease may appear in the horse and it would prove of very real interest and of vital importance to the surgeon if an equine form of thrombo-angiitis obliterans were disclosed, supported by clinical and histological details. Work on these lines might result in the determination of a food factor as the cause and in this respect certain features common to thrombo-angiitis obliterans and poisoning by ergot are at least suggestive.

REFERENCES.

- ¹Buerger, L. (1924). *The Circulatory Disturbances of the Extremities*. W. B. Saunders Company, Philadelphia and London.
- ²Hutyra and Marek. (1926). *Special Pathology and Therapeutics of the Diseases of Domestic Animals*. 3rd American Edition. 2. p. 830.

REVIEWS.

Handbook for Veterinary Surgeons. By Fred Bullock, LL.D., Barrister-at-Law. Third Edition. 1936. London: Baillière, Tindall & Cox. Price 7s. 6d.

It is very satisfactory to learn that Dr. Bullock's valuable work has reached a new edition, not only because it indicates a possible reward to the author for his labours, but more because it affords evidence that increasing numbers of veterinary surgeons and veterinary students are recognising that it is for them indispensable.

The new edition gives evidence of careful revision and it has been considerably enlarged. The matter has also been to some extent rearranged with a view to making it more helpful to Final-year Students who are preparing for the examination in veterinary jurisprudence, in accordance with the syllabus approved by the Royal College of Veterinary Surgeons.

The reports of proceedings in Courts of Law too frequently show that some veterinary witnesses would have escaped humiliation if they had made themselves well acquainted with the chapter headed "Procedure in Courts of Law." But, indeed, the book is packed with information which every member of the profession ought to make himself familiar.

Bernhard Bang. Selected Works. Edited by Vald. Adersen, Professor at the Royal Veterinary and Agricultural College, Copenhagen. Copenhagen: Levin & Munksgaard. London: Humphrey Milford, Oxford University Press. 1936. Price 30s.

This work owes its origin to the desire of Dr. Bernhard Bang's fellow-countrymen to commemorate his memory and to give a measure of permanence to his teaching regarding a number of important diseases to the investigation of which his life was devoted. The matter contained in it is not original, but consists of reprints of papers which appeared in different journals and in different languages during the author's life-time. They are concerned with Actinomycosis, Mastitis in Cattle, Local Bacillary Necrosis, Swine Erysipelas, Contagious Abortion, Johne's Disease, Tuberculosis of the Cow's Udder, Experimental Researches regarding Tubercle Bacilli in Milk, the Destruction of Tubercle Bacilli through Heat, the Importance of Koch's Tuberculin for the Diagnosis of Tuberculosis in Cattle and Swine, Tuberculin and its Employment in Combating Tuberculosis in Cattle, Congenital Tuberculosis in Calves, The Prevention of Animal Tuberculosis, The Struggle against Tuberculosis by National Measures according to the Sources of Infection, The Importance of the Detection of the Tubercle Bacillus in the struggle against Tuberculosis in the Domesticated Animals. The papers appear in English, French or German, but not always in the language of the original paper. Three of the articles are in French, ten in English and thirteen in German.

Bang was a prolific author but nothing that he wrote was ephemeral and most of his teaching is likely to be of permanent value. All his papers to an exceptional degree inspire confidence because he never wrote except on the basis of wide personal knowledge of the subject. It is to be hoped and expected that veterinary teachers everywhere will continue to be fully informed concerning Bang's original work, and this splendid volume will make that possible.

It extends to 560 Imp. octavo pages, clearly printed on beautiful paper. There is a life-like photo-portrait of Bang taken in 1920 and a short preface by Prof. Vald. Adersen.

One learns from the preface that a short time before Prof. Bang's death it had been suggested to him that a volume containing the most important of his works should be published, but that the negotiations concerning this suggestion had not been completed at the time of his death. At the request of the Publisher Professor Adersen undertook to prepare the volume, and that was made possible by a grant from the Rask-Ørsted Foundation. Great credit is due to Prof. Adersen and to all who collaborated with him in the work.

Bang's researches were concerned mainly with diseases of animals, but, as everyone knows, those relating to tuberculosis, and especially those concerning tuberculosis of the cow's udder and tubercle bacilli in milk, are of equal interest to the medical profession, and it is to be hoped that this volume will be widely read in both professions.

